NMR quantitative analysis - The application of lanthanide shift reagents.

Kwakye, J. K.

Award date:
1979

Awarding institution:
University of Bath

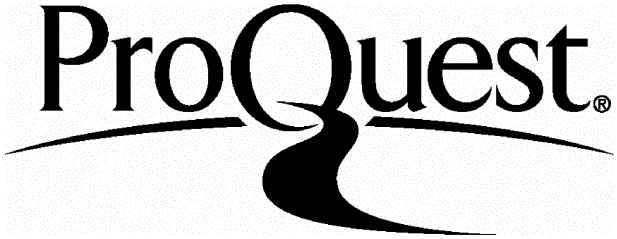
NMR QUANTITATIVE ANALYSIS - THE APPLICATION OF LANTHANIDE SHIFT REAGENTS

THESIS

Submitted by J.K. KWAKYE, B. Pharm., M.Sc.
for the Degree of Ph.D.
of the UNIVERSITY OF BATH

1979

This research has been carried out in the Department of Pharmaceutical Chemistry, University of Bath.



To the memory of my father

R.Y. Gyening

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor R.T. Parfitt and Dr. G.H. Dewar for their moral support, guidance and supervision throughout the course of this work.

I would also like to express my gratitude to Professor R.Sibson of the Mathematics Department, University of Bath for providing the mathematical treatment on the 'base line technique' which is given in the appendix.

My thanks go to Mr. Roland R. Hartell, Chief Technician in the Department of Pharmaceutical Chemistry, for his devotion in the search for chemicals and apparatus and also for his general help in matters pertaining to my welfare.

I wish also to thank the staff of the School of Pharmacy, University of Bath, for their friendliness.

I would like to express gratitude to the following drug companies: ICI, Ltd.; May and Baker; Allen and Hanburys Ltd; Nicholars Laboratories; Janssen Pharmaceutic, Belgium; Boots Company; Winthrop Laboratories; John Wyeth and Brother Ltd.; Lake and Cruickshank Ltd.; Syntex Laboratories Inc., California,and Lilly Research Centre Ltd.; for their generous supply of samples for this work.

The successful completion of this work has been partially due to the moral support and encouragement from my wife. I would like to express my appreciation to her for all that she has done to make this

work complete.

Finally, I would like to thank Mrs. J. Harbutt for typing the manuscript.

SUMMARY

Though NMR has many applications in analytical chemistry, it has not been widely used for quantitative analysis. This has been due partly to high instrumental cost and relatively poor sensitivity. With advances in instrumentation and the development of new techniques, the full potential of nmr for quantitative studies is now being investigated.

The use of nmr for quantitative analysis has been reviewed with particular reference to pharmaceuticals. The basis of the nmr quantitative analysis and the factors affecting the suitability of the technique have been outlined. The theory and application of lanthanide shift reagents have been considered with emphasis on optical purity determination.

Chiral lanthanide shift reagents have been used successfully to determine enantiomeric ratio of many optically active compounds. Three methods, namely, the base line technique, chemical shift difference and peak height difference methods have been developed to overcome the problem of accurate measurement of broad resonance peaks normally associated with the use of lanthanide shift reagents. The application of the base line technique has been extended to non-enantiomeric compounds.

Modification of structures of some compounds is sometimes necessarry before the application of lanthanide shift reagents. An account of compounds which need modification has been given.

The increasing number and complexity of pharmaceutical preparations encounted in routine analysis of drugs requires the application of techniques that are rapid, specific and accurate. In this thesis nmr as a quantitative tool for pharmaceuticals is fully investigated. Many pharmaceuticals of different dosage forms have been assayed successfully by the use of nmr.

A colorimetric method has been used for the kinetic studies of 'Benoral' suspension, a preparation containing benorylate (4-acetamidophenyl 2-acetoxybenzoate). The suspension has been found to be quite stable despite the two ester groups in the benorylate molecule.

TABLE OF CONTENTS

Page

Page

1. PART I - INTRODUCTION

Advances in both instrumentation and application of nuclear magnetic resonance (nmr) have arisen with remarkable speed following the discovery of chemical shifts in 1951[1] which set the stage for the use of nmr as a probe into the structure of molecules. Before this discovery, nmr was the domain of the physicist and was used largely for the elucidation of fundamental nuclear phenomena and the accurate determination of nuclear magnetic moments. The impact of the technique on analytical chemistry is evidenced by the number of chemical articles that have appeared in the literature over the past few decades. Many of these are based on the application of nmr to structure elucidation of new compounds. It is probably the most powerful tool available for structure elucidation. Two of the recent advances on the applications of nmr are the use of nmr for quantitative analysis of pharmaceuticals[2] and, more recently, the discovery of Lanthanide shift reagents which often simplify spectra[3] and allow optical purity determinations[4].

1.1 Quantitative Pharmaceutical Analysis

Nuclear magnetic resonance is a technique particularly suited to quantitative measurements but the pharmaceutical applications were not appreciated until 1963 when Hollis successfully analysed Aspirin, Phenacetin and Caffeine mixtures (APC) by nmr spectroscopy[2].

The application of nmr in quantitative analysis depends on the fact that the area beneath a particular nmr signal is directly proportional to the number of protons from which the signal is derived so that the area under a $\underline{CH}_3$ signal is thrice larger than that under a $\underline{CH}$ signal of the same molecule. The area (A) under a signal is

given by

$$A = K\frac{W}{E} \qquad \ldots.(1:1)$$

Where 'W' is the weight of the sample

'E' is the equivalent weight of the compound which is given by

$$E = \frac{\text{Molecular weight of the compound}}{\text{the number of protons giving that signal}}$$

and 'K' is a constant.

Application of equation (1) in quantitative analysis requires at least two signals, one from the test sample and the other from a standard sample (see page 5 for an account of the standards employed). The area (A_T) under the analytical signal for the test sample is given by

$$A_T = K\frac{W_T}{E_T} \qquad \ldots.(1:2)$$

Similarly for the standard signal, the area (A_S) is given by

$$A_S = K\frac{W_S}{E_S} \qquad \ldots.(1:3)$$

Dividing equation (1:2) by equation (1:3) gives the weight of the test sample (W_T) as

$$W_T = \frac{A_T E_T W_S}{A_S E_S} \qquad \ldots.(1:4)$$

Both peak height and area measurements are used in quantitative nmr analysis but it is the latter technique which is most often used, because it is only under certain conditions that peak heights are proportional to the number of protons giving rise to the signal, namely if the line widths are identical[5]. The peak height of the absorption mode of the nmr signal under slow passage conditions is given by

$$I = K\frac{\gamma H_1 N}{t}\quad\frac{T_2}{(1+(\gamma H_1)^2 T_1 T_2} \qquad \ldots.(1:5)$$

where 'N' is the number of nuclei and 't' the absolute temperature. The dependence of the peak height on T_2, which is a measure of the line width, and on the effects of spin-spin coupling, limits its usefulness in quantitative work. The area of the signal under the same conditions is given by

$$A = \frac{K'\gamma H_1 N}{t} \quad \frac{1}{(1+(\gamma H_1)^2 T_1 T_2} \qquad \ldots.(1:6)$$

For sufficiently low H_1 levels, $(\gamma H_1)^2 T_1 T_2 \ll 1$ and the area is approximately given by

$$A = \frac{K'\gamma H_1 N}{t} \qquad \ldots.(1:7)$$

In this case the relative areas of the signals in a spectrum are directly proportional to the numbers of nuclei giving rise to them. Peak heights are, however, preferred to area measurement of signals from very dilute solutions[6], and also in the case of measurement in the proximity of other signals from the sample matrix or solvent[7].

There are several ways of measuring the area under a signal but the most commonly employed method is by integration using electronic integrators which are available for most nmr spectrometers. The height of the step of the integral curve (I_T) is proportional to the area (A) under the peak.

$$I_T = KA \qquad \ldots.(1:8)$$

where 'K' is a constant which depends on the sweep rate (R). Thus,

$$I_T = \frac{A}{R} \qquad \ldots.(1:9)$$

Other methods of area measurements include triangulation, cutting and weighing[5], and by means of a planimeter[8].

The accuracy of area measurement and the degree to which an area truly represents the number of protons giving rise to the integrated signal depends critically upon the following instrumental operating conditions:

(a) the signal-to-noise ratio, which must be high to avoid background noise contribution to the integration trace;

(b) a non-saturating mode of operation by a proper choice of radio-frequency (rf) power level (H) and sweep rate (dH/dt); (At high H_1 levels a significant degree of saturation occurs, and since the relaxation times are not equal for all signals there is no longer a simple relationship between signal areas and numbers of nuclei. Both slow and rapid sweep rates are unsuitable for normal quantitative work. Sweep times for slow passage are unacceptably long, especially when measurements have to be repeated. On the other hand, sweep rates for rapid passage lead to considerable broadening, and cannot be used for measurements of signals which are close together. Intermediate sweep rates are therefore used, and conditions have to be selected such that relaxation times do not affect relative peak areas[9]);

(c) reduction of spinning side bands by careful adjustment of magnetic field homogeneity and use of high precision sample tubes spinning at optimum rate;

(d) correct settings of both base line zero and rf phase control to give true absorption mode of operation and to avoid drifting or slope in the integral curves.

To minimise errors due to integration, analytical resonance signals

must be integrated several times (normally five times) and the average taken.

The use of an internal standard is a common practice in nmr quantitative analysis. The choice of internal standard is critical and depends upon several factors, including solubility and compatibility[5, 10]. The standard and the test analytical peaks should not coincide, and also the two peaks should have comparable areas to minimise errors caused by non-linearity of the electronic measuring circuits[8]. The material should be readily available[11] and should give a single sharp resonance[5, 11] (this condition is not always met). Among the most common compounds used as standards are; 1,4-dioxan[12], tetramethyl ammonium bromide[11], biphenyl-or benzyl benzoate[10], hexamethylcyclotrisiloxane[13,14], succinamide[15], *t*-butanol[16], maleic acid[17], malonic acid[6], methanol[6], fumaric acid[5], maleic anhydride[5], and cyclohexane[5]. Solvent ^{13}C satellites and residual protons in deuterated solvents have also been used as internal standards[5].

Though solvents used in ^{1}H nmr spectroscopy should be devoid of protons; this is not always possible and other proton-containing solvents have been used. The essential requirement is that the solvent should have a transparent spectrum in the analytical region and, in addition, the sample and standard must show acceptable solubility and compatibility with the solvent. For ordinary quantitative analysis, solvents commonly used include carbon tetrachloride, deuterated chloroform, tetrachloroethylene, carbon disulphide, deuterated water, deuterated acetic acid and trifluoroacetic acid[10, 11, 18]. Combinations of solvents can be used. For

example, in the analysis of methenamine and methenamine mandelate tablets, a mixture of formamide, acetone and acetonitrile (10 + 25 + 65) was used as the solvent[17]. The solvent system was selected to resolve problems of solubility of methenamine and its salt-methenamine mandelate, overlapping of the resonance signals of the components and potential decomposition of methanamine.

To overcome solubility and incompatibility problems, the standard can be placed in a separate tube as an external standard. An analytical technique for the determination of a molecular species in a mixture employing an external standard in a precision coaxial tube has been developed[19]. The method has also been used for the elemental determination of hydrogen, and for some binary mixtures by the measurement of chemical shift differences.

Since Hollis' Aspirin, Phenacetin and Caffeine (APC) mixtures analysis in 1963, several reports of successful nmr analysis of pharmaceuticals have been published[5-57]. In 1964 nmr spectroscopy was used for the characterization and quantification of detergent chemicals[20]. For alkylbenzenes, alkylphenols, and ethylene oxide (EO) adducts of alkylphenols, the following quantities can be measured: average lengths of the alkyl chains, average molecular weights, degree and kind of branching in the alkyl chain, *ortho-para* distribution of aryl substituents, and average lengths of EO chains. The procedure is simple. It involves integration of two major resonance bands in the nmr spectrum belonging to the aromatic protons (lower field) and the protons of the alkyl chain (higher field). Since the lower band results from exactly five protons

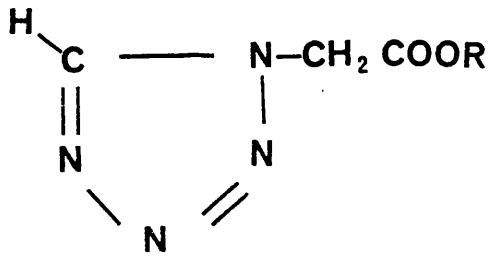
(in the case of alkylbenzenes) per molecule, assuming only mono-alkylation, the average number of hydrogens in the alkyl chain, the average carbon number of the alkyl chain, as well as the average molecular weight of the sample can be readily obtained from the relative integrals of the two bands. Accuracy is of the order of ±2% of the total hydrogen. A combination of nmr and mass spectrometry has been used to examine the purity of some non ionic surfactants[21].

The importance of silicones in the pharmaceutical, cosmetic and food industries has given rise to various chemical and physical methods for the identification and quantitation of this material. A simple nmr assay method for dimethylpolysiloxane (dimethicone) coating in needles of syringes has been presented by Anhoury *et al.*[12] This involves the use of tetrachloroethylene as the solvent and 1,4-dioxane as the internal standard. The silicone on metal needles cut from syringes is extracted with a mixture of tetrachloroethylene and 1,4-dioxan (99.6:0.4, v/v) and the nmr spectra obtained on 0.5 ml samples of the solution. The 1,4-dioxan signal at δ3.54 ppm and the silicone $\left[(CH_3)_2Si<^{O-}\right]$ signal at δ0.1 ppm are used as the basis for the analysis. This nmr method compares favourably with the usual IR method which depends on analysis of the absorbance peak at 1090 cm^{-1}.

Although a number of methods exist for the determination of hydroxyl groups in simple mixtures and petroleum oils, a more reliable and independent method is still needed to correlate the total hydroxyl content and the nature of the hydroxyl moiety. Recently, an nmr method has been described by Schweighardt *et al.*[22] for the character-

ization and quantitation of hydroxyl groups in complex organic mixtures by formation of their trimethyl silyl (TMS) derivatives. A series of model compounds was used to demonstrate the ninefold enhancement in proton nmr sensitivity, which subsequently gave a calculated hydroxyl content with an average error of 5%. This study also showed the chemical shift of the trimethylsilyl group protons in each TMS derivative formed to vary, in general, with the nature of the hydroxyl group - alcohols and benzylic hydroxyls being the most shielded and polynuclear aromatic carboxylic acids and oximes least. The method was then applied to the characterization of hydroxyl groups found in coal liquefaction oils and asphaltenes. The use of TMS ether derivatives in nmr quantitation is also reported for fatty acids[23] and hydroxyl in coal[24].

To determine quantitatively the relative isomeric ratios of 1H (1:1A)- and 2H(1:1B)-Tetrazole-1-acetic acids and their ester analogues, it was necessary to use a specific method. A fast and accurate nmr method has been described by Staiger *et al.*[25]. The method takes advantage of the significant difference in chemical shift of the methylene groups or the ring protons in the 1- and 2-tetrazole acetic acid and analogues. The relative ratio of the isomers is



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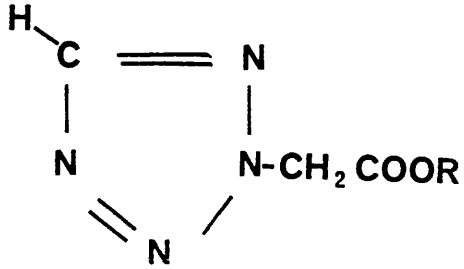
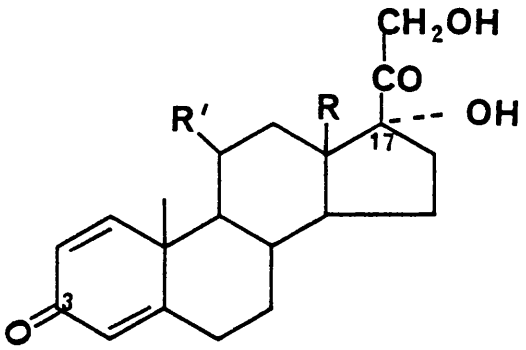


(1:1B)

determined from the measured intensities of the methylene groups. The importance of this analysis is that the presence of the 2-isomer can lower the purity of the desired pharmacologically active material.

The official[27, 153] assay procedure for synthetic corticosteroids of the 1,4-dien-3-one type (1:2A) is by colourimetric methods based on the reduction of certain tetrazolium derivatives[173] by the α-ketol side chain at C-17. Such methods are not specific since they do



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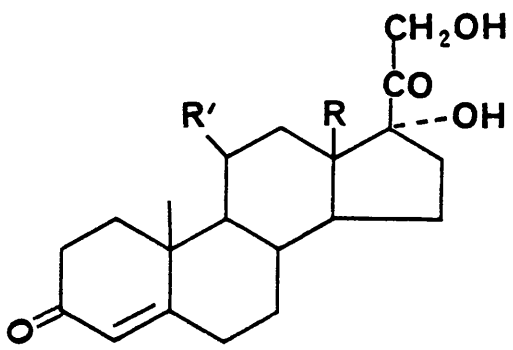
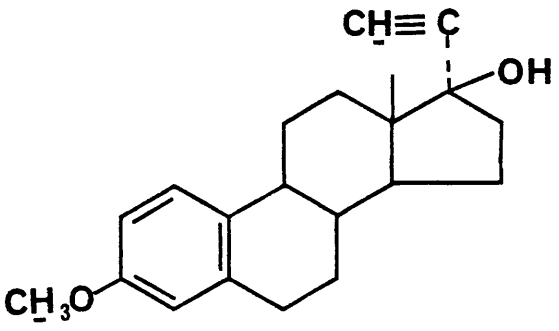


(1:2B)

not distinguish between 1,4-dien-3-ones and related corticosteroids of the 4-en-3-one (1:2B) type, which may be present as impurities. NMR spectroscopy affords a method of distinguishing easily between the two groups of steroids. 1,4-Dien-3-ones possess three vinylic protons of which the chemical shift usually differs from that of the single vinylic proton of 4-en-3-ones. It is, therefore, possible to detect the two groups of compounds in the presence of each other, and hence to develop a much more specific assay procedure. Avdovich *et al.*[28] have described an nmr method for the analysis of synthetic corticosteroids of the 1,4-dien-3-one type. Dimethyl sulphoxide and fumaric acid were employed as the solvent and the internal standard

respectively. Both bulk drugs and formulations were assayed using this method. Comparisons were made with results obtained from official assays on the steroids and their formulations. The average deviation obtained with the nmr method was 0.6%. A procedure for the water-soluble 1,4-dien-3-ones (such as Prednisolone sodium phosphate (P.S.P)) was also described using triethylamine hydrochloride and D_2O as the internal standard and solvent respectively. The signals at 7.58δ (for PSP) and 3.22δ (for triethylamine hydrochloride) were used in the analysis.

An nmr assay procedure has also been developed for Mestranol (1:3)[29]. The method is based upon measurement of the nmr signals belonging to the methoxy and ethinyl groups using diphenylacetic

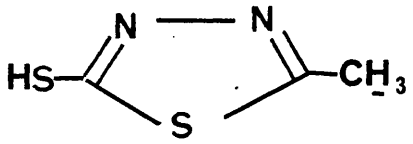


(1:3)

acid as an internal standard and pyridine as the solvent.

NMR quantitative analysis has generally been accomplished with the use of an internal standard. However, free thiadiazole (1:5) in cefazolin (1:4) has been quantified by nmr spectroscopy using a calibration curve derived from spectral data on known mixtures prepared from pure thiadiazole and pure cefazolin (sodium salt or free acid)[30]. The method takes advantage of the difference

of the chemical shift of the methyl protons on the free thiadiazole



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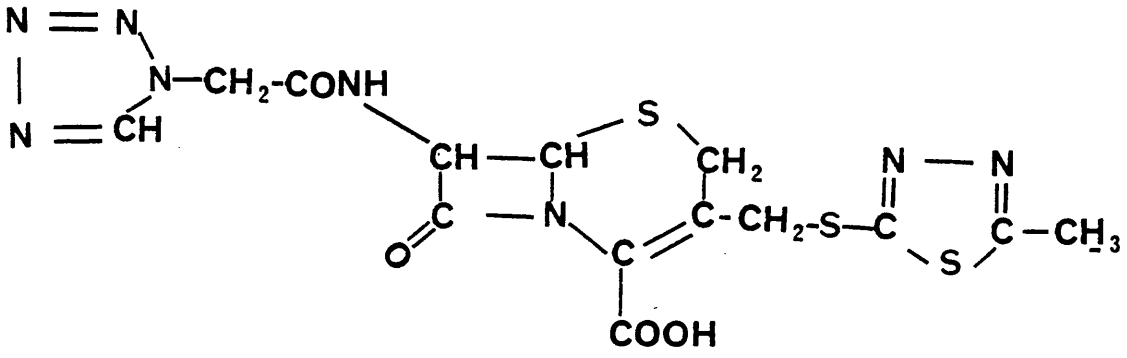
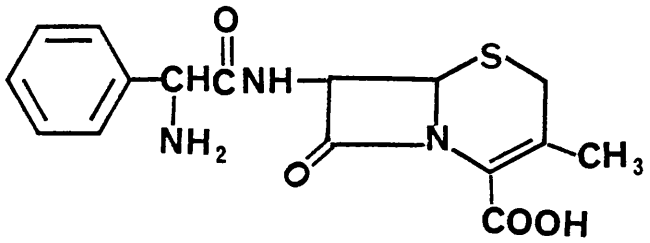


(1:4)

and the thiadiazole moiety of cefazolin; the nmr signals appear at δ2.54 and δ2.84 ppm respectively. The authors claim that this technique avoids the potential weighing errors inherent in the use of an internal standard.

Cephalexin (1:6A) may be present in a given lot of cephradine (1:6B) as an impurity from the synthesis of (1:6B) or as a decomposition product of(1:6B). An nmr method to determine quantitatively the presence of cephalexin in cephradine has been reported by Warren *et al.*[175] The method is applicable to the chemical itself as well as to capsules and oral suspension formulations. The determination is based on the nmr signal from the five aromatic protons of cephalexin molecule. The precision at the 2% cephalexin



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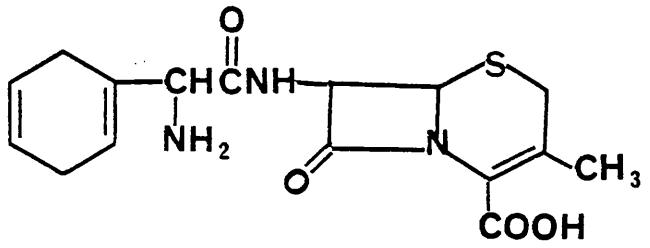
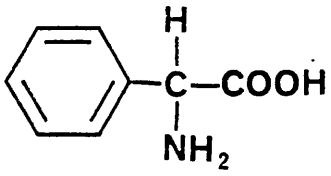


(1:6B)

level is ±0.18%. The time required to carry out a single analysis is about 10 mins, and five analysis can be done in about 0.5 hr.

Another example of the use of nmr in quantitative analysis is reported[11] for a four-component mixture of phenylglycine derivatives. A rapid, accurate, and precise nmr analytical method for the analysis of phenylglycine (1:7A), dihydrophenylglycine (1:7B), tetrahydrophenylglycine (1:7C) and cyclohexylglycine (1:7D) in combination with each other has been developed. The method is based on the integration of the nmr signal characteristic of each component relative to the signal from tetramethyl-ammonium bromide, which is added as an internal standard (see Table 1:1). No prior separation of



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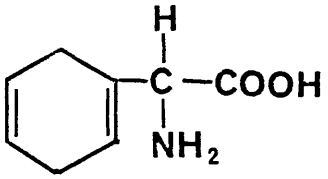

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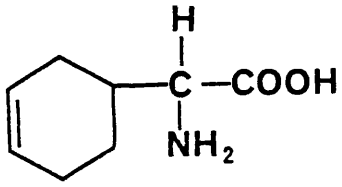

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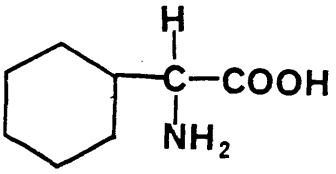


(1:7D)

the four components is required. 2% DCl solution was used as the solvent.

Table 1:1[11] Chemical shifts of signals characteristic of individual components.

Component	Chemical shift (δ)	Number of protons
Phenylglycine	7.60	5
Dihydrophenylglycine	2.84	4
Tetrahydrophenylglycine	1.92	5
Cyclohexylglycine	1.25	5
Tetramethylammonium bromide	3.36	12

The analysis of several other pharmaceuticals is listed in Table 1:2, page 15. All authors of published reports advocate that the nmr method has an accuracy, precision, and sensitivity comparable to the existing methods. The method offers the advantage over others of being rapid and specific.

Most of the analysis is based on the selection of a part of the spectrum where there is no overlap of signals. The method is limited to only those compounds or preparations whose signals do not overlap in the entire spectrum. This means that only pure single compounds and certain mixtures can be analysed by this method. Moreover, apart from protons in close proximity to electronegative centres or those attached directly to Sp^2 hybridized carbon atoms, which give rise to resonance in characteristic low field positions, most other resonances occur in the $\delta 0.5 - 2.5$ ppm region. This gives rise to coincident peaks from formally non-equivalent protons and non-analysable spectra[59]. Many of these problems can be solved by the use of lanthanide shift reagents. Other techniques generally available include spectrum simplification by irradiation of the samples with a second radiofrequency (a spin-decoupling experiment) and variation of the ambient probe temperature, but these methods have not been applied in the quantitative analysis of pharmaceutical products[49]. Larger magnets (higher rf powers) simplify spectra and resolve peaks close together. However, this advantage is offset by the high cost of the instruments. Spectra may often be simplified by removing interfering O<u>H</u> and N<u>H</u> proton signals through addition of a few drops of heavy water (D_2O) or gaseous acid vapour[49]. The use of D_2O as a solvent eliminates the interfering N<u>H</u> and O<u>H</u> signals.

Table 1:2 Analysis of some pharmaceutical preparations

Preparation	Internal Standard	Standard Deviation	Reference
Chloral Hydrate in soft Gelatin Capsules	maleic acid	0.6%	32
Trimethadione in various dosage forms	*t*-butanol	±0.3%	33
Methsuximide and Phensuximide in capsules	HMCTS	0.6% or less	14
Acetazolamide and its sodium salt in various dosage forms	*t*-butanol	0.73%	16
Thiotepa	benzoic acid	0.6%	31
Carbromal and Bromural in Tablets	HMCTS	Carbromal=0.7% Bromural=0.5%	13
Amyl Nitrite in Inhalant dosage form	biphenyl- or benzyl-benzoate	±0.5	10
Pentylenetetrazol in tablets and injectables	HMCTS	0.7%	34
DMSO in solutions and ointments	methanol	0.7%[a]	6
Meprobamate in tablets	malonic acid	0.9%	35
Polydimethylsiloxane in various dosage forms	Hexamethyl-benzene or *p*-dichlorobenzene	0.2	36
Glutethimide in tablets	HMCTS	±0.97	37
Cacodylate injections	succinic acid	±2.3%	38
Aminophylline in tablets	*t*-butanol	0.5%	39
Disulfiram in tablets	HMCTS	1.3%	26,40

[a]Average relative error

HMCTS = hexamethylcyclotrisiloxane

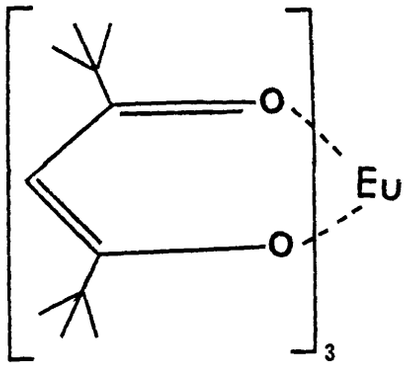
Small sample size has been a problem in nmr quantitative analysis due to the relatively low sensitivity of nmr spectrometers. The use of a computer of average transients (CAT) and glass micro cells has, to a large extent, solved this problem. For CAT a spectrum is scanned repeatedly a preselected number of times. The signal output from the spectrometer is stored and accumulated by the computer which presents a final spectrum with a total signal proportional to the number of passes through the spectrum and with the noise accumulated as the square root of the number of passes. This gain in signal to noise ratio and the build up of sample signal produces an nmr spectrum on microgram quantities of sample. The technique has been used for the determination of 0.1 mg of barbiturate mixtures and for the analysis of 0.05 mg gas liquid Chromatography (g.l.c.) fractions from a commercial terpene fraction[50]. CAT has also found application in biological systems[51].

Another problem with nmr quantitative analysis is the possible interference from the drug excipients, ^{13}C satellites and spinning side bands. Fortunately cases of interference from drug matrix are said to be rare[49]. Most of the exipients are insoluble in the solvents used for the analysis and are therefore filtered off. Spinning side bands can often be eliminated by careful adjustment of the sample spinning rate. The usual spectrum of $^{13}C\underline{H}$ consists of one large peak flanked on both sides by a small one due to the ^{13}C coupling with the proton. Since the natural abundance of ^{13}C is 1.108%, each of the small peaks has 0.55% of the intensity of the large one. It is therefore possible to correct for the ^{13}C satellites. A procedure for the correction has been reported[2, 8].

Reviews on the use of nmr in pharmaceutical analysis have been presented by Rackman[48, 49], Casy[41], Parfitt[9, 42] and Philipsborn[43]. A book on nmr quantitative analysis by Kasler[5] has been published. A vivid account of the applicability of nmr spectroscopy to regulatory drug analysis has been given by Kram and Turczan[44]. Articles by Alexander and Koch[45-47] are also relevant to nmr quantification. Many other articles on nmr quantitative analysis have been reported. These include analysis of quinidine and hydroquinidine[52, 53], amyl nitrite[54], *p*-hydroxybenzoates[55], diglycerides[56] and barbiturates[57].

1.2 Lanthanide Shift Reagents

Spectral simplification by the use of solvents[18] and transition metal complexes as shift reagents have long been observed but these were of no use to the analytical chemist because their induced shifts were too small to be of any practical value. Moreover, due to the relatively slow electron spin relaxation times of the transition metal ions, line broadening[60] resulted in the loss of signal multiplicity from which much of the structural information is derived[61]. The real break-through came in 1969 when Hinckley[3] discovered that the addition of the pyridine adduct of Trisdipivalomethanato europium (lll) $[Eu(DPM)_3]$ (1:8) to a solution of cholesterol in carbon tetrachloride caused a downfield shift in the spectrum of the steroid. He found also that shifts for protons close to the point of association were



(1:8)

larger than for those protons further removed and there was an absence of line broadening effects.

The findings of Hinckley stimulated the interest of many workers and in 1970 Saunders and Williams[62] prepared a pyridine-free complex of (1:8) and found that the absence of pyridine increased the shifts in the spectrum of cholesterol four-fold. It is considered that pyridine functioned as a competitive inhibitor for association with the substrate which decreased the induced shift.

1.2.1 Mechanism of Lanthanide Induced Shift

The chemical shift change ($\Delta\delta$) caused by addition of a paramagnetic species can be divided conveniently into three terms[61,63,64,65]:

$$\Delta\delta = \Delta\delta(\text{contact}) + \Delta\delta(\text{dipolar}) + \Delta\delta(\text{diamagnetic})$$

The contact interaction involves direct delocalization and/or spin polarization of the unpaired electron *via* the molecular orbitals of the substrate ligand. As a result, the unpaired electron spin density is spread over a number of atomic sites in the ligand, thereby inducing a contact shift (i.e. through covalent bond formation). Coupling between the electron and nuclear spins does not cause observable splittings because of rapid relaxation of the electron spin. The field experienced by the nucleus being observed is a weighted average for opposite orientations of the electron spin. This is not zero since the thermal populations of the electronic energy levels are not equal. The resultant shift can be to high or to low field, depending on the sign of the electron-nuclear coupling constant. Since contact shifts are caused by the transmission of unpaired electron spin density through bonds, they are observed most extensively

in ligands with delocalized π systems. In δ-bonded systems they are rapidly attenuated with increasing separation from the metal ion.

The dipolar, or Pseudocontact shift, arises from secondary magnetic effects generated by the magnetic moment of the paramagnetic ion, and are transmitted through space. The induced pseudocontact shift (Δδ) of an axially symmetric molecule is expressed by the following equation (McConnell-Robertson equation[66]).

$$\Delta\delta \text{ (dipolar)} = \frac{x(3\text{Cos}^2\theta - 1)}{r^3} \qquad \ldots\ldots(1{:}10)$$

where 'r' is the distance vector joining the metal cation to the particular nucleus (Fig. 1:1), 'θ' is the angle between that vector (r) and the line connecting the metal ion and the co-ordinating atom (N) (i.e. the principal symmetry axis), so that θ is the H-Ln-O angle for the protons in an alcohol; 'x' is a constant for any given metal adduct at a given temperature and is dependent on the magnetic anisotropy of the ion.

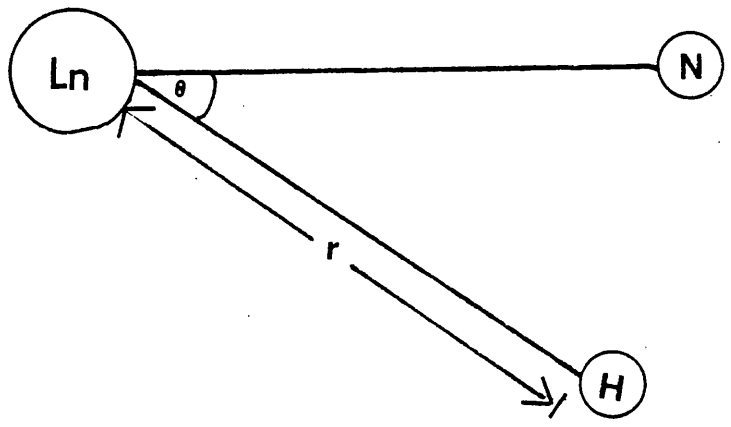


Fig. 1:1 Co-ordination of Lanthanide ion with a compound.

The pseudocontact shift is clearly a powerful source of structural information provided that it can be separated from other contributions to the observed shifts.

The third term (diamagnetic) encompasses all factors not resulting from the paramagnetism of the metal ion. While this term is frequently much smaller than the others, it cannot be ignored if accurate values of the pseudocontact shift are being sought. The Lanthanide shift reagents produce their effects mainly through the pseudocontact shift mechanism[67]. However, contributions from contact interaction is possible, particularly for protons attached to the carbons nearest the lone pair bearing atoms[64]. Contact shifts depend on the overlap of orbitals containing unpaired electrons with ligand orbitals. For the transition metal ions with unpaired d electrons, contact shifts are sometimes several kilohertz. The unpaired f electrons of lanthanide complexes are in compact orbitals shielded from ligands, so that contact interactions are smaller or even absent. Contact shifts are primarily a source of information about electronic structure whereas pseudocontact shifts can be related to geometrical structure. Thus, through the use of lanthanide induced shifts molecular geometry can be elucidated.

1.2.2 Factors affecting the lanthanide (pseudocontact) induced shift

The magnitude of the induced shift ($\delta\Delta$) depends on several factors:

(a) Temperature: The effect of temperature on lanthanide induced shifts has been extensively studied[70]. Generally, a decrease

in temperature increases the magnitude of the shift. Armitage and Hall[176, 177] were the first to report increased LIS at lower temperatures. Dependence of LIS on the inverse of the absolute temperature have been observed over the temperature range - 30 to 90°C[61], but it has been stated that in any single molecule all the protons may not always respond uniformly to temperature changes[70, 116]. Bennett and Schuster have reported a case where an increase in temperature increases the LIS (Fig. 1:2)[71].

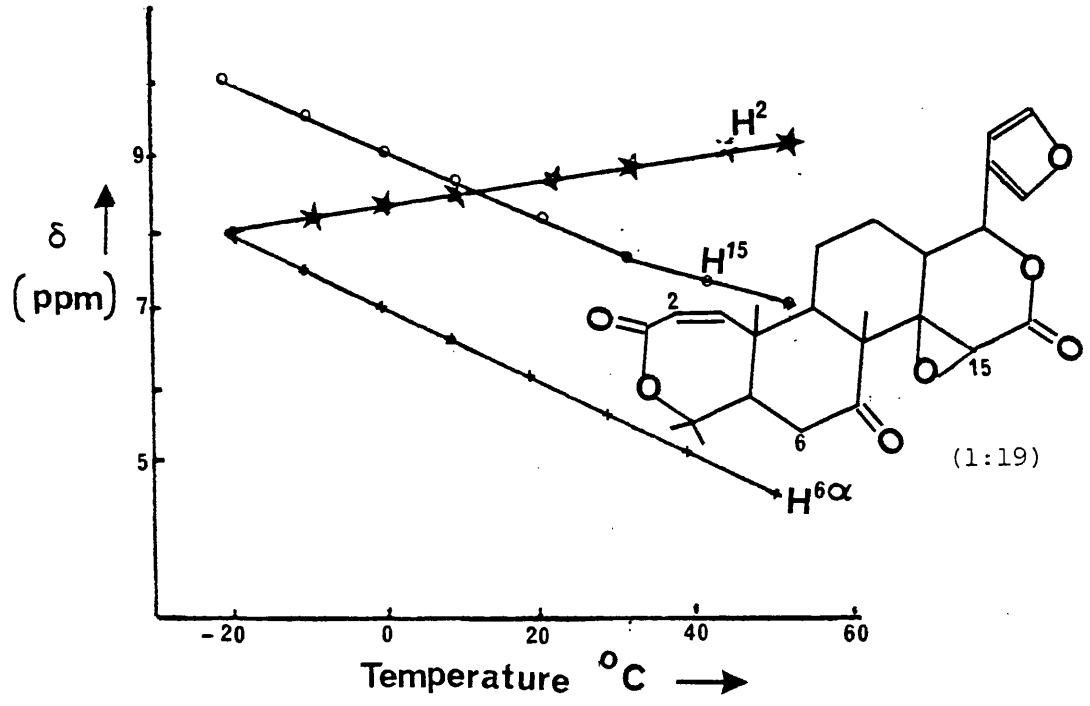


Fig. 1:2 LIS of three protons of Obacunone (1:19) [Eu(fod)$_3$/ substrate = 0.6] as a function of temperature[71].

(b) Distance: The nearer a proton is to the site of co-ordination, the greater the shift. An excellent illustration to this point is given by shifts observed in the n-heptanol (1:9) spectrum after addition of Eu(dpm)$_3$[64]. The order of the

$$\underset{A}{HO} - \underset{B}{CH_2} - \underset{C}{CH_2} - \underset{D}{CH_2} - \underset{E}{CH_2} - \underset{F}{CH_2} - \underset{G}{CH_3}$$

(1:9)

induced shifts was as follows:

A > B > C > D > E > F > G

(c) The Geometric term ($3Cos^2\theta-1$): The angle term in equation (1:10) determines the direction of the shift. Generally, Eu shifts downfield and Pr upfield. However when θ is between 125.3° and 54.7°, the induced shifts will be in the opposite direction to the normal shifts, e.g. upfield for Eu and downfield for Pr[61,64]. This reversal in the direction of the shift corresponds to the term $3Cos^2\theta-1$ becoming negative for this range of angles. This accounts for the occasional observation that as one adds a shift reagent such as $Eu(fod)_3$ (1:10E), most peaks are shifted downfield, but some are shifted upfield or remain unchanged[68]. The shift reversal has been observed for protons in cases where the substrate envelopes the metal ion[67].

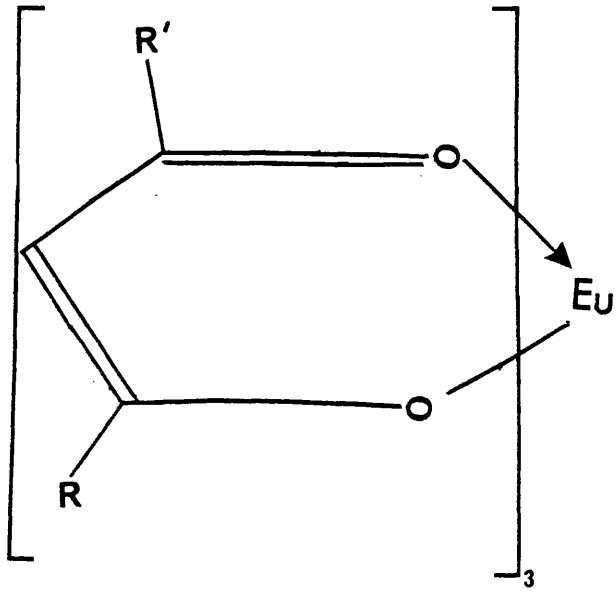
(d) Ratio of shift reagent to substrate: At low concentration of lanthanide shift reagent, a linear concentration dependence of the induced shift is observed which is used to control the magnitude of the shift.

1.2.3 The Shift Reagents

The Lanthanide ion forms a complex with an organic molecule to form the Lanthanide shift reagent (LSR). The most useful complexes are β-diketone derivatives. These are often air stable and soluble in organic solvents, and have very simple nmr spectra. The LSR consists of a six co-ordinate metal complex which readily expands its co-ordination number in solution by accepting further ligands.

These complexes behave as Lewis acids, binding basic substrates as labile ligands. Owing to the magnetic interactions with the metal ion in the complexed substrate, the nmr positions of the associated nuclei in the complex differ from those in the uncomplexed state. The equilibrium in solution between these species is rapid on the nmr time scale so that only a single average signal is recorded for each nucleus in the different environments (an exception is the use of $Eu(fod)_3$ with dimethyl sulphoxide in deuteriomethylene chloride at $-80^{\circ}C$ in which slow chemical exchange is reported[64]). These shift reagents complex with a wide variety of substrates and the complexes are soluble in most of the solvents normally used for nmr. The solutions are stable but decomposition is caused by compounds with acidic groups such as carboxylic acids and some phenols. In these cases suitable derivatives must be prepared[65].

Several LSRs are now available. Each LSR has advantages and disadvantages depending on the substrate used and other factors. However, it appears that those prepared from Europium (Eu) are often superior to the others. Even though LSRs prepared from other metals of the lanthanide block (Ln^{3+}) may cause greater shifts than Eu, they are of limited application because of their serious line broadening. Praseodymium (Pr) shift reagents are the next most superior LSRs. Their shifts are larger than those caused by europium, compensating for line broadening. They induce upfield shifts which make them useful complementary reagents. A disadvantage of Praseodymium and other shielding LSRs, however, is the added complication of crossing-over of resonances, which confuses analysis in some cases.



(1:1O)

1:1OA R = R' = CH_3

1:1OB R = R' = C_6H_5

1:1OC R = CF_3; R' = CH_3 - C(CH_3)(CH_3)

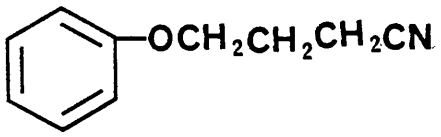
1:1OD R = CF_3CF_2; R' = CH_3 - C(CH_3)(CH_3)

1:1OE R = $CF_3CF_2CF_2$; R' = CH_3 - C(CH_3)(CH_3)

The following are some examples of Europium-β-diketone chelates (their structures are given on page 24): Tris-(acetylacetonato) europium (111) $Eu(acac)_3$ (1:1OA); Tris-(dibenzoylmethanato) europium (111) $Eu(dbm)_3$ (1:1OB); Tris-(1,1,1-trifluoro-5,5-dimethylhexane-2,4-dionato) europium (111) Eu(fhd) (1:1OC); Tris-(1,1,1,2,2,-pentafluoro-6,6-dimethyl heptane-3,5-dionato) europium (111) Eu(pfd) (1:1OD); Tris-(1,1,1,2,2,3,3,-heptafluoro-7,7-dimethyloctane-4,6-dionato) europium (111) $Eu(fod)_3$ (1:1OE).

By virtue of their Lewis acidity and their solubility in common nmr solvents, the fluorinated derivatives of the LSRs give larger shifts because of the stronger binding in the complex. The greater the number of fluorine atoms in the molecule, the larger the induced shift. Thus, the order of the shifting power of the above mentioned LSRs is $Eu(fod)_3 > Eu(pfd)_3 > Eu(fhd)_3 > Eu)dbm)_3$. The extent of the induced shift also depends on the nature of the functional group in the substrate. Greater shifts are caused by functional groups which are most basic[64]. Functional groups give characteristic shift magnitudes in the order, $NH_2 > OH > C = O > CHO > -O- > CO_2R > -CH$[59]. This order reflects the dissociation constant of the corresponding substrate-reagent adduct and, to a smaller extent, the geometry (distance and angle) of the protons with respect to the central metal-ion[70]. Imines, azobenzenes, halides and nitro compounds are not affected by LSRs[59, 69]. In the case of functional groups consisting of two possible donor atoms, co-ordination is thought to occur mainly with the oxygen atom when present. For example, amides co-ordinate at the oxygen atom[64].

Where a substrate contains more than one functional group it is obvious that co-ordination with the LSR will occur at the most basic centre. However, some co-ordination at the other centres is also likely[72]. Where the most basic centre is attached to a bulky group, steric hindrance prevents the co-ordination at that centre. The greatest shift in 4-phenoxy butylnitrile (1:11) is obtained from the methylene group $\underline{\alpha}$ to the cyano group, indicating that the complex is formed through the nitrile and not the oxygen atom[72].



(1:11)

Lanthanide chelates are very hygroscopic and should be stored over a suitable desiccant (e.g. P_2O_5) *in vacuo*. On absorption of water, the chelates usually become white solids and their shifting power is drastically reduced. The anhydrous $(dpm)_3$ chelates should have the following colours[61]: pale green (Pr), white (Sm, Tb, Yb, Gd, Dy, Tm, Lu), Pale yellow (Eu, Ho), red (Ce), violet (Nd), and pink (Er).

As in all nmr analyses, the choice of solvent is important. Here, the solvent should be capable of solubilizing the chelate, the substrate, and the complex formed between the two without interacting preferentially with the chelate. Thus, for Ln $(dpm)_3$ and Ln$(fod)_3$, solvents with strong Lewis base properties (e.g. ROH, R_2SO) are precluded. CCl_4 and $CDCl_3$ are the most common solvents employed. For substrates which are insufficiently soluble in non-

polar solvents, lanthanide salts can be used. The induced shifts when these reagents are used in polar solvents are very small because the solvent itself may co-ordinate to the reagent. Lanthanide salts, notably the hydrated chlorides, nitrates and perchlorates, have been used as shift reagents in such solvents. The induced shifts tend to be smaller than those observed with the β-diketonates because hydration reduces the acidity of the lanthanide ion[73]. Solvents which have been used include DMSO-d, D_2O, deuteroacetone, deuteromethanol and deutero pyridine[61, 65]. Europium trichloride has been used in dimethyl sulphoxide to study polyfunctional steroids[74]. With the hydrated lanthanide ions, most shifts induced by europium are to high field and those by praseodymium are to low field whereas the reverse is the case with the β-diketonate complexes (see page 22). For quantitative structural studies the salts have some disadvantages compared with the β-diketonate complexes. As the lanthanide ion is not surrounded by bulky ligands, contact shifts are likely to be more important.

Tricyclopentadienyl lanthanide complexes are an additional class of shift reagent[75]. They are relatively strong acids and form stable and soluble 1:1 adducts with a variety of donors. The induced shifts in these adducts may be very large (40 to 50 ppm). Unfortunately the complexes are extremely sensitive to air and water, which limit their usefulness.

Lanthanide shift reagents have helped solve a variety of structural problems including structure elucidation, detection and estimation of isomeric impurities[76], conformational analysis,

separation of overlapping signals, absolute configuration of closely related compounds[193] and the sign determination of spin-spin coupling constants[77]. The chiral derivatives of the LSRs are used for the direct optical purity determination of mixtures of enantiomers.

1.2.4 Chiral Lanthanide Shift Reagents - Determination of Optical Purities of Enantiomers

Enantiomers are identical in all respects except in the spatial arrangement (chirality) of the atoms attached to the optical centre. Thus, they have identical physical and chemical properties (except that they have equal but opposite optical rotations). Their nmr spectra are also identical in non-chiral solvents.

The optical purity of a substance is the ratio of its specific rotation (α) to the specific rotation (A) (Absolute rotation) of the pure enantiomer.

$$\text{Optical purity} = \frac{\alpha}{A} \qquad \ldots.(1:11)$$

$$\% \text{ optical purity} = \frac{100\ \alpha}{A} \qquad \ldots.(1:12)$$

Optical purity has the same value as the enantiomeric purity which is defined as the measure of the excess of one enantiomer over the other. Thus

$$\text{Enantiomeric purity} = \frac{l-d}{l+d} \qquad \ldots.(1:13)$$

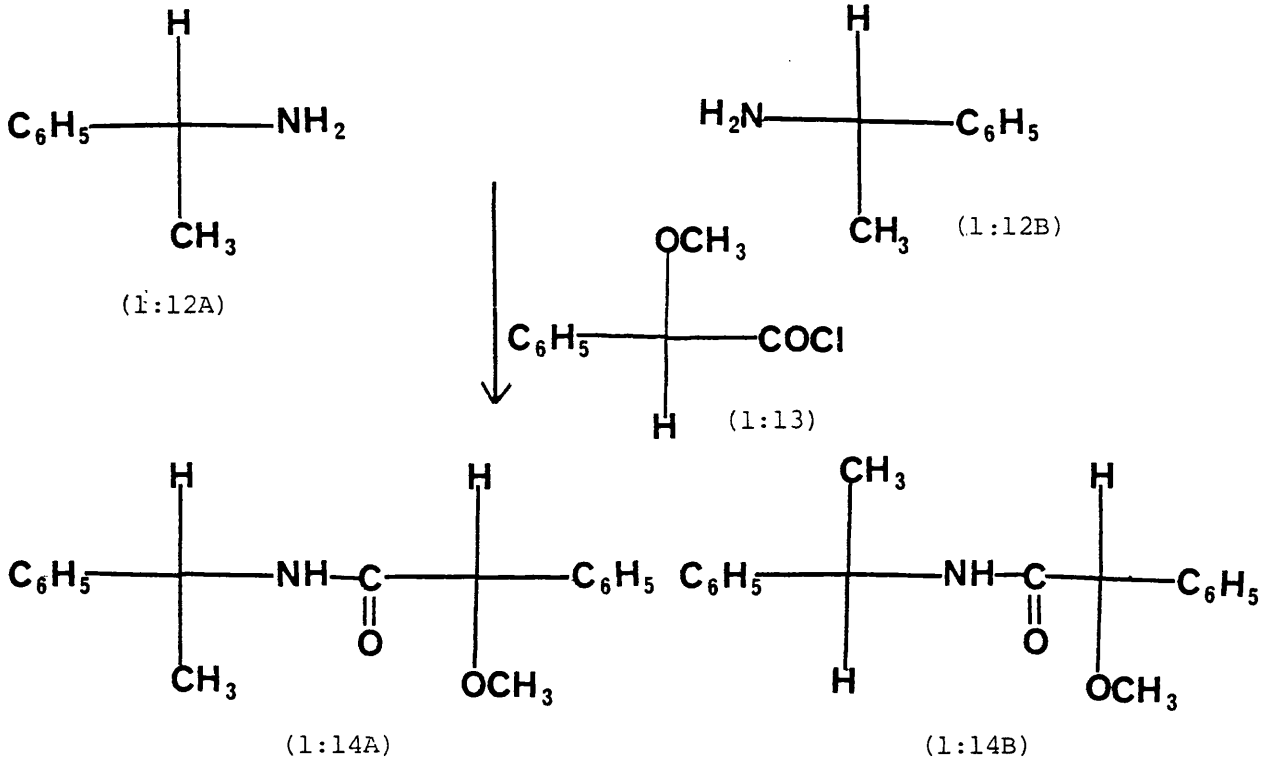
$$\% \text{ Enantiomeric purity} = \frac{100(l-d)}{l+d} \qquad \ldots.(1:14)$$

where 'l' and 'd' are the mole fractions of the enantiomers.

The most common methods for optical purity determinations include polarimetry, which measures the optical rotation (α) of the compounds, chromatography, particularly GLC[78-84], and more recently HPLC[88-91], and nmr, the most recent technique. Other methods, less extensively employed, include isotope dilution[85], kinetic resolution[86, 87], and calorimetric methods[92]. The nmr method has been described as the most convenient and simple way to determine enantiomeric purity[93].

Before the advent of chiral lanthanide shift reagents (CLSR), two methods were used to determine the optical purities of enantiomers by nmr. The first one was through the formation of diastereoisomers and the second through the use of chiral solvents. Diastereoisomers have different physical properties and hence different nmr spectra. In principle, the optical purities are determined by linking the enantiomers to optically pure enantiomorphs of a suitable reagent and examining the nmr spectrum of the total diastereoisomeric mixture[8]. For the method to be successful, chemical shift differences between diastereoisomers must be sufficiently pronounced and care must be taken to ensure quantitative reaction of both enantiomorphs (so that the diastereoisomers ratio exactly reflects the enantiomeric ratio) and also that no racemisation or equilibrium process occurs. These two requirements are met by the use of an excess of optically pure chiral reagents and the correct sequence of the reaction process. A substantial literature is available on nmr determination of optical purity by the method of diastereoisomer formation, particularly by Raban and co-workers[94,95,96]. Jacobus and Raban describe the determination of the optical purity

of α-phenylethylamine (1:12A) and (1:12B) by converting it into a mixture of diastereomeric amides (1:14A and 1:14B) with optically pure *O*-methylmandeloyl chloride (1:13). The two c-methyl groups (as well as the other corresponding groups) in (1:12) are enantiotopic and consequently have the same nmr chemical shift (the signals from the groups are isochronous). By contrast, the c-methyl groups (as well as the other corresponding groups) in (1:14) are diastereotopic and as a result have different nmr chemical shifts (the signals from the groups are anisochronous).

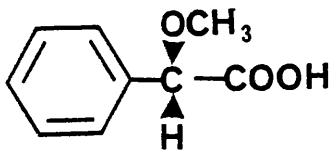


Scheme 1:1

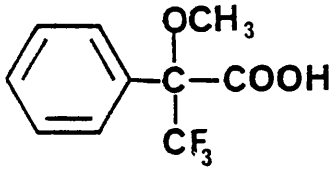
The relative intensities of any of these sets of signals are in proportion to the relative abundances of the two diastereoisomers, and the ratio of the integrated intensities (R) can be used to calculate the optical purity (OP) using the following equation:

$$OP\ (\%) = \frac{R-1}{R+1} \times 100 \qquad \ldots.(1:15)$$

(R)-*O*-methylmandelic acid (1:15) has been used as a chiral derivatising agent for optical purity determination of secondary carbinols[97]. Another reagent, α-methoxy-α-trifluoromethylphenyl-acetic acid (1:16), introduced by Mosher[98], has many advantages over (1:13) and (1:15). Mosher's reagent (1:16) generally exhibits excellent



(1:15)



(1:16)

separation of diastereotopic fluorine and proton resonances, marked stability to racemisation of derivatives under severe conditions, and the presence of a trifluoromethyl group permits the use of fluorine nmr. This reagent has been applied to the determination of optical purity of a series of secondary amines and alcohol[98], phenylethylene glycol[99], and phenyltrimethylsilylcarbinol[100].

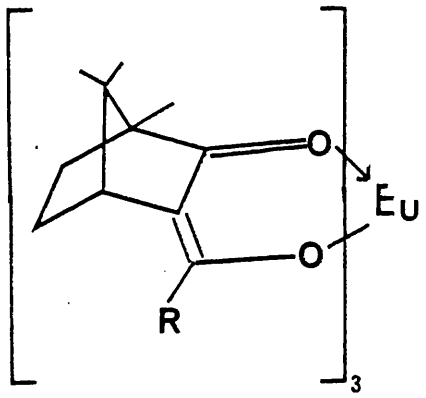
The second method of nmr optical purity determination is based on the principle that enantiomers give non-identical nmr spectra in chiral solvents. Thus, diastereomeric interactions are involved rather than with diastereomeric compounds. When a racemic solute (E_+, E_-) is dissolved in a racemic solvent (S_+,S_-), transient diastereotopic solvates are formed. Since solute-solvent interactions are averaged on the nmr time scale, the diastereomeric

interactions in the two enantiomeric solvates sets $E_+S_+ - E_+S_-$ and $E_-S_- - E_-S_+$, produce only one set of solute signals. However, in an optically active solvent (S_+) only one set of diasteriomeric interactions (E_+S_+ and E_-S_+) exists and enantiotopic nuclei of the solute are then unequally screened. These nuclei, in principle, should show different chemical shifts, and integration of their resonances would then provide a direct measure of the enantiomeric purity of the solute. This method has been exployed extensively by Pirkle and co-workers[101,102]. Pirkle and Beare[101] determined the enantiomeric S:R ratio of α-amino acid methyl esters by observing their nmr chemical shift differences in optically active 2,2,2-trifluorophenylethanol. This solvent has been the most useful reagent for a variety of amines, esters and sulphur and phosphorus compounds. An important feature of this technique is that enantiomeric configurations can be correlated with chemical shift differences and thus absolute configurations can be established[179-181]. However, the method is limited to polar solvent-solute combinations because non-equivalence results from diastereomeric solute-solvent interactions. Shift differences tend to be small (< 0.04 ppm) which also limits the usefulness of this technique[103]. Optically active nmr Lanthanide shift reagents give a greater induced shift.

1.2.4.1 The use of Chiral Lanthanide Shift Reagents (CLSRs)

Whitesides and Lewis[4] first observed that enantiomeric amines dissolved in achiral solvents containing 3-(*t*-butylhydroxymethylene)-d-camphorato europium (111) (1:17A) gave relatively large frequency differences between corresponding resonances (compared with the two previous methods described above). The observations indicated that

(1:17A) and related compounds (1:17A-D) could provide the basis for a useful method for determining the enantiomeric purity of certain compounds for which procedures based on diastereomeric interaction between solute and solvent fail. In practice the utility of (1:17A) is restricted to applications involving relatively basic and unhindered substrates (e.g. primary and secondary amines), and fails with less basic substances. A large number of chiral shift reagents have been synthesised which demonstrate the general applicability of these materials to the determination of the enantiomeric purity of relatively non-basic substances. As with the ordinary lanthanide shift reagents, the halogenated derivatives of the chiral LSRs are superior to the non-halogenated ones[49, 104, 105]. In addition to fluorination, the potential of chiral shift reagents can be improved by increasing the size of the chelate group[61].



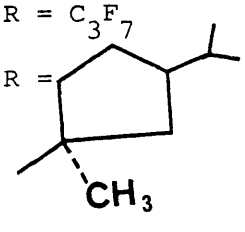
(1:17)

1:17A R = $C(CH_3)_3$

1:17B R = CF_3

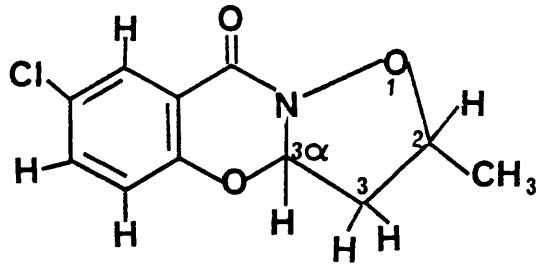
1:17C R = C_3F_7

1:17D R =



Shifts over 1 ppm have been observed using the fluorinated CLSRs[58]. By proper choice of shift reagent and conditions, shift differences for at least one set of enantiotopic protons are often large enough and sufficiently separated from other signals to allow enantiomeric compositions to be determined directly from relative peak areas.

A simple nmr method was developed by Reisberg, Brenner and Bodin[106] for the determination of the enantiomers of 7-chloro-3,3α-dihydro-2-methyl-2H, 9H-isoxazolo [3,2,-b] [1,3] benzoxazin-9-one (1:18) using (1:17C). The $Eu(hfbc)_3$ causes the doublet assigned to the protons of the 2-methyl group, which normally appears at



(1:18)

about 1.5 ppm (δ), to split into two pairs of doublets and to shift downfield to about 2.0 - 3.5 ppm. The downfield pair of doublets (2.5 - 3.0 ppm) re presents the two enantiomers present in one racemate, designated as the β form, while the upfield pair (2.0 - 2.5 ppm) represents the enantiomers of the racemate designated as the α-form. From the integration of the area under the doublets, the relative concentration of all four enantiomers was determined. It is interesting to note that with $Eu(fod)_3$ the methyl group of both α- and β-forms of (1:18) appears as a pair of doublets in the 2.5 - 3.5 ppm region, but the doublets are not split into two

pairs as seen with CLSRs[107]. The hfbC (1:17C) chelates have been found to induce larger shifts than the facam (1:17B) chelates. However, shift differences for enantiomers are not consistently larger for a particular reagent. The magnitude of non-equivalence vary with the shift reagent: substrate ratio in unpredictable ways[104]. The properties of these CLSRs are similar to the ordinary LSRs.

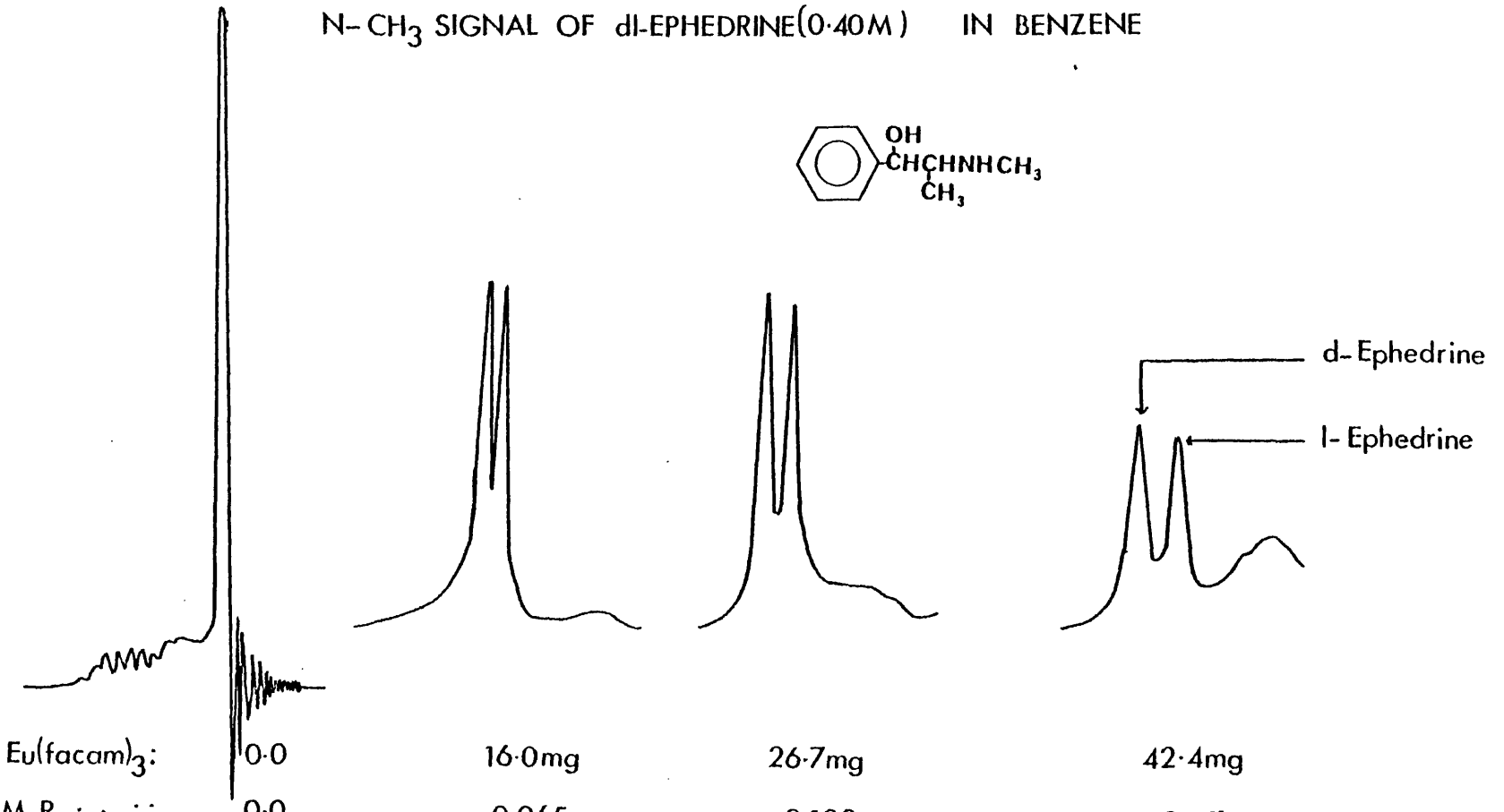
Accurate knowledge of optical purities is important in the manufacture of pharmaceutical products where the presence of one enantiomer in the drug might render the product toxic or of modified pharmacological activity and also in the area of polypeptide synthesis , where a small amount of optical impurity in the monomeric starting material can lead to a polymeric product of low homogeneity[92]. It is therefore essential to have a simple, fast and accurate method of determining optical purities. CLSRs afford a powerful means of analysing optical purity and offer a much more convenient alternative to the previous methods.

PART 2

2. OPTICAL PURITY (ENANTIOMERIC RATIO) DETERMINATION EMPLOYING CHIRAL LANTHANIDE SHIFT REAGENTS, AND THE APPLICATION OF THE BASE LINE TECHNIQUE TO NON-OPTICALLY ACTIVE COMPOUNDS.

2.1 GENERAL DISCUSSION

FIG.2.1 EFFECT OF INCREMENTAL ADDITION OF $Eu(facam)_3$ ON N-CH_3 SIGNAL OF dl-EPHEDRINE(0·40M) IN BENZENE



$Eu(facam)_3$:	0·0	16·0mg	26·7mg	42·4mg
M.R. : : ::	0·0	0·065	0·108	0·171
$\Delta\Delta\nu$: : : :	0·0	0·07 ppm	0·12 ppm	0·17ppm
ν : : :	2·1ppm	1·94ppm	1·84ppm	1·75ppm

UPFIELD SHIFT →

At low molar ratios, incremental addition of a chiral lanthanide shift reagent (CLSR) induces a differential shift proportional to the amount of shift reagent. As seen in figure 2.1 (page 36) incremental addition of Eu(facam)$_3$ to dl-Ephedrine (2:3) in benzene (d_6) almost completely separates the *N*-CH_3 signals belonging to the d- and l-forms. It is possible to achieve complete separation of the two isomers [as in the case of tetramisole (Section 2.2.5)], but in most cases complete separation is not achieved for the following reasons:

(1) Broadness of the resonance peaks with increase in reagent concentration;

(2) Saturation at the co-ordination centre;

(3) Merging of the resonance signals under study with other resonance signals, either from the same molecule or from the reagent;

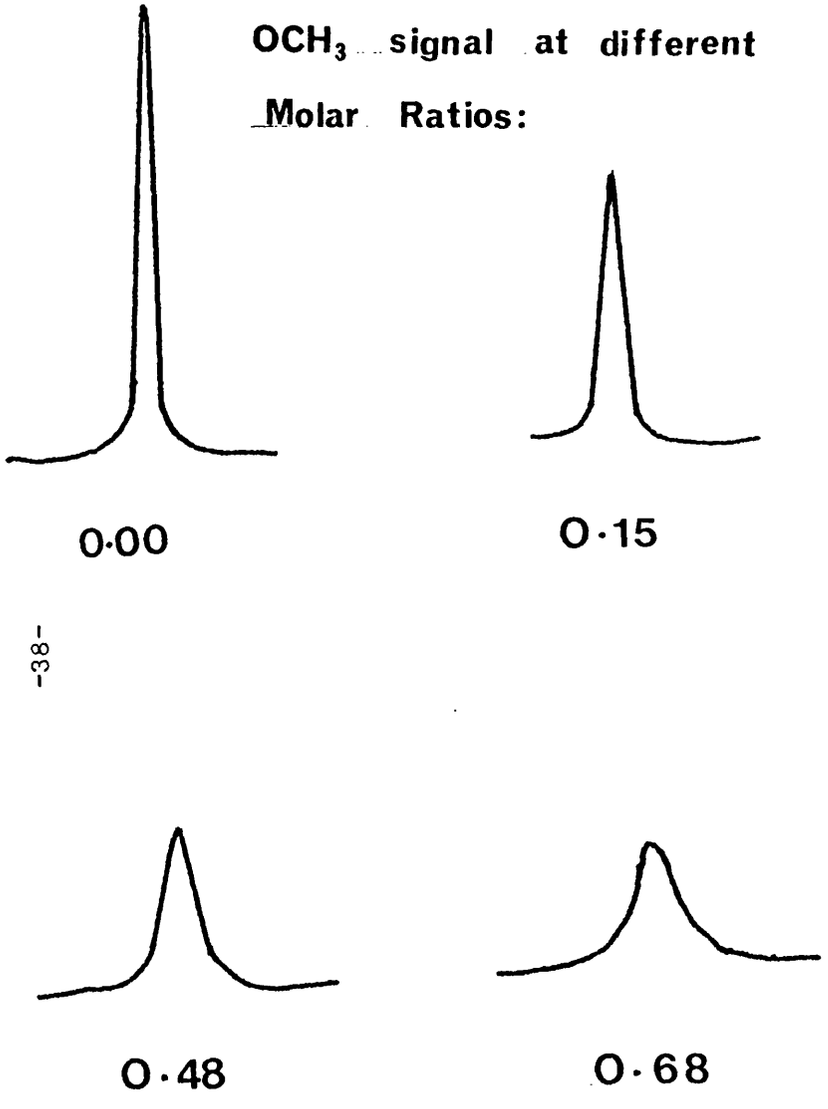
and (4) Insolubility of the shift reagent in the solvent at high reagent concentration.

The first factor is the most important.

The use of lanthanide shift reagents is almost always associated with broadness of the resonance peaks. In all the compounds studied, a plot of the broadness (the width at ½ height) against the molar ratio (Reagent / Substrate) gave a linear relationship. A typical plot of such a relationship is represented in figure 2.2 (page 38) using the results obtained from Propranolol (2:5). The extent of the broadening depends on how close the particular proton group under study is to the co-ordination centre. This broadness often precludes

FIG.2.2

RELATIONSHIP BETWEEN BROADNESS AND MOLAR RATIO



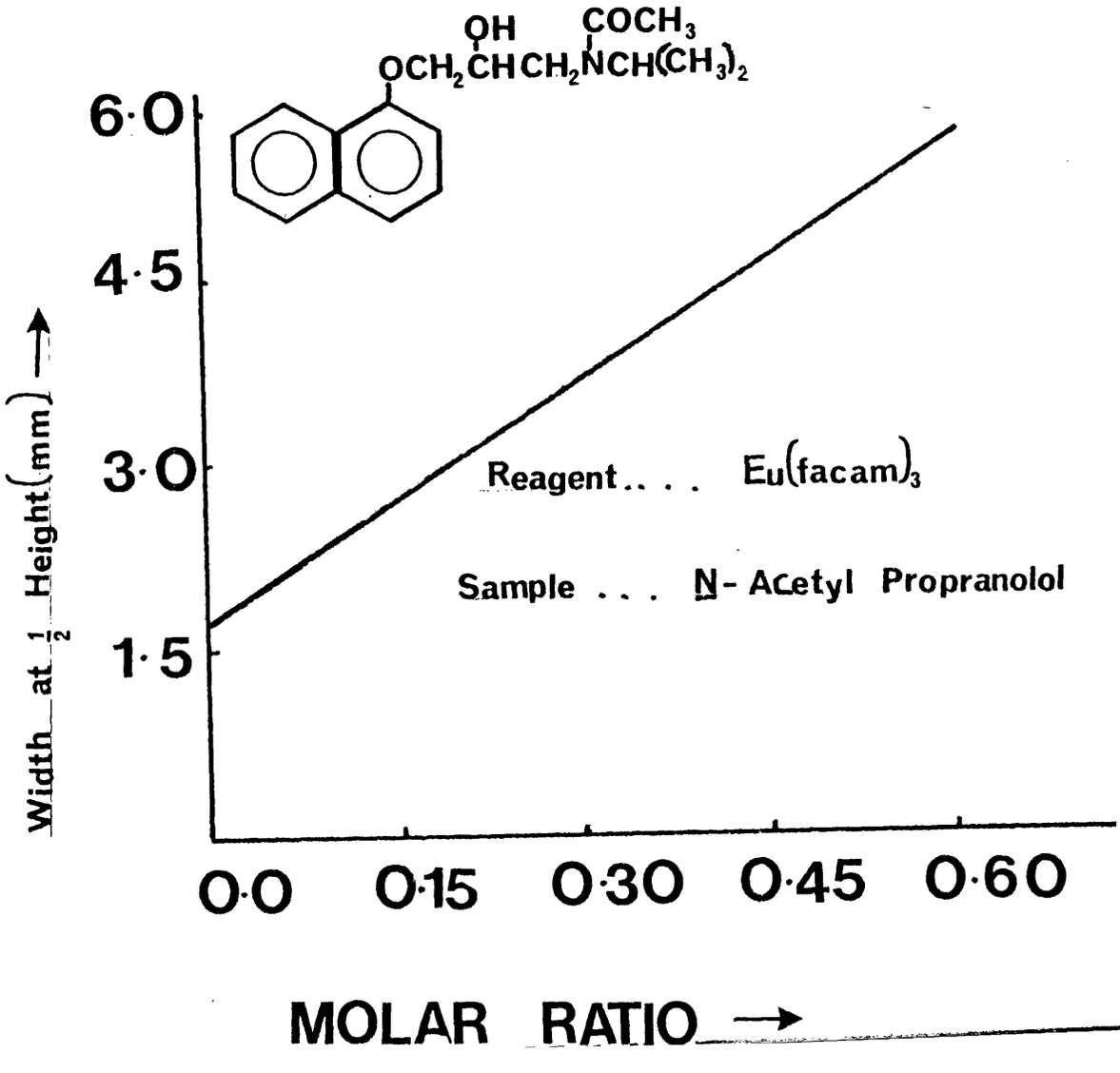
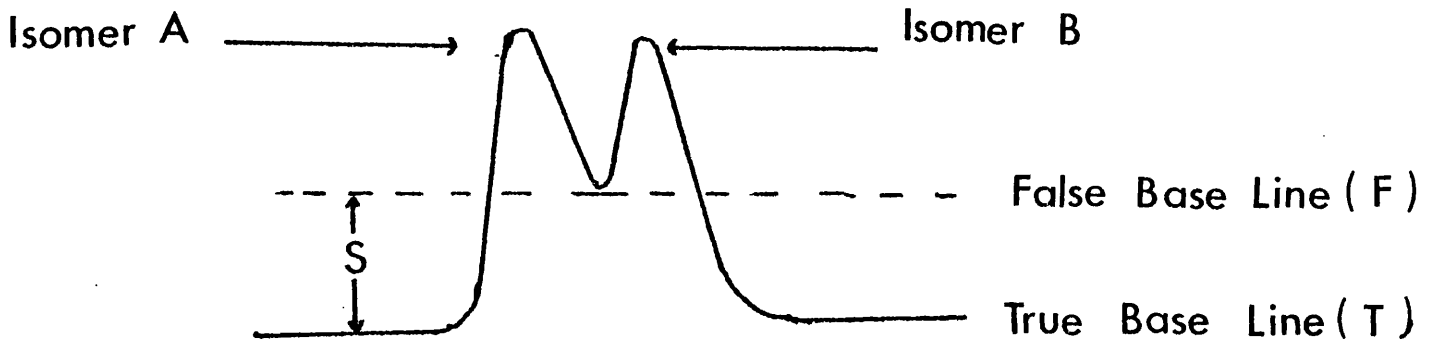


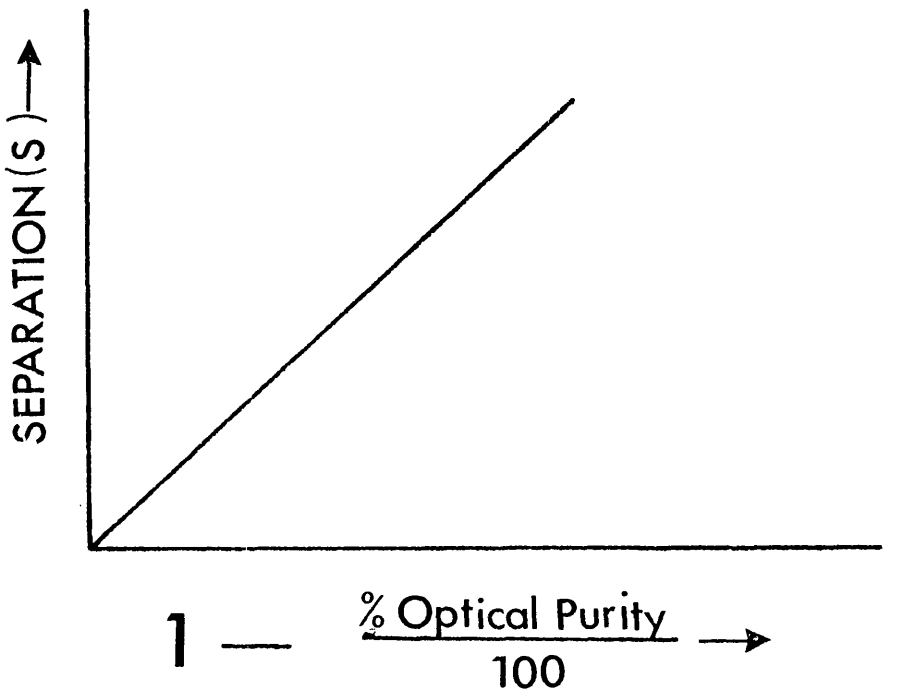
FIG. 2.3

THE BASE LINE TECHNIQUE



F — T designated as SEPARATION (S)

$$S \propto 1 - \frac{\%\ \text{Optical Purity}}{100}$$



complete separation of the two signals belonging to the two isomers, and integration is not feasible. Three techniques have been devised to overcome this problem:

(i) The base line technique;

(ii) The chemical shift difference (δv) method;

(iii) The peak height difference (δh) method.

(i) The base line technique

The 'base-line' technique involves a consideration of two nmr bands assigned to the same functional group of the enantiomers ((+) and (-)) under examination and which are not fully resolved in the presence of a chiral lanthanide shift reagent. This is illustrated in figure 2.3 (page 39). The distance (S) between the base line (i.e. the true base line T) and the trough between the bands (false base line F) is proportional to 1 - % optical purity (OP)/100 at a constant reagent to substrate molar ratio. Therefore, a plot of the separation (S) versus 1 - OP/100 gives a linear relationship which can be used to estimate the enantiomer ratio.

The successful application of this technique depends upon three major conditions:

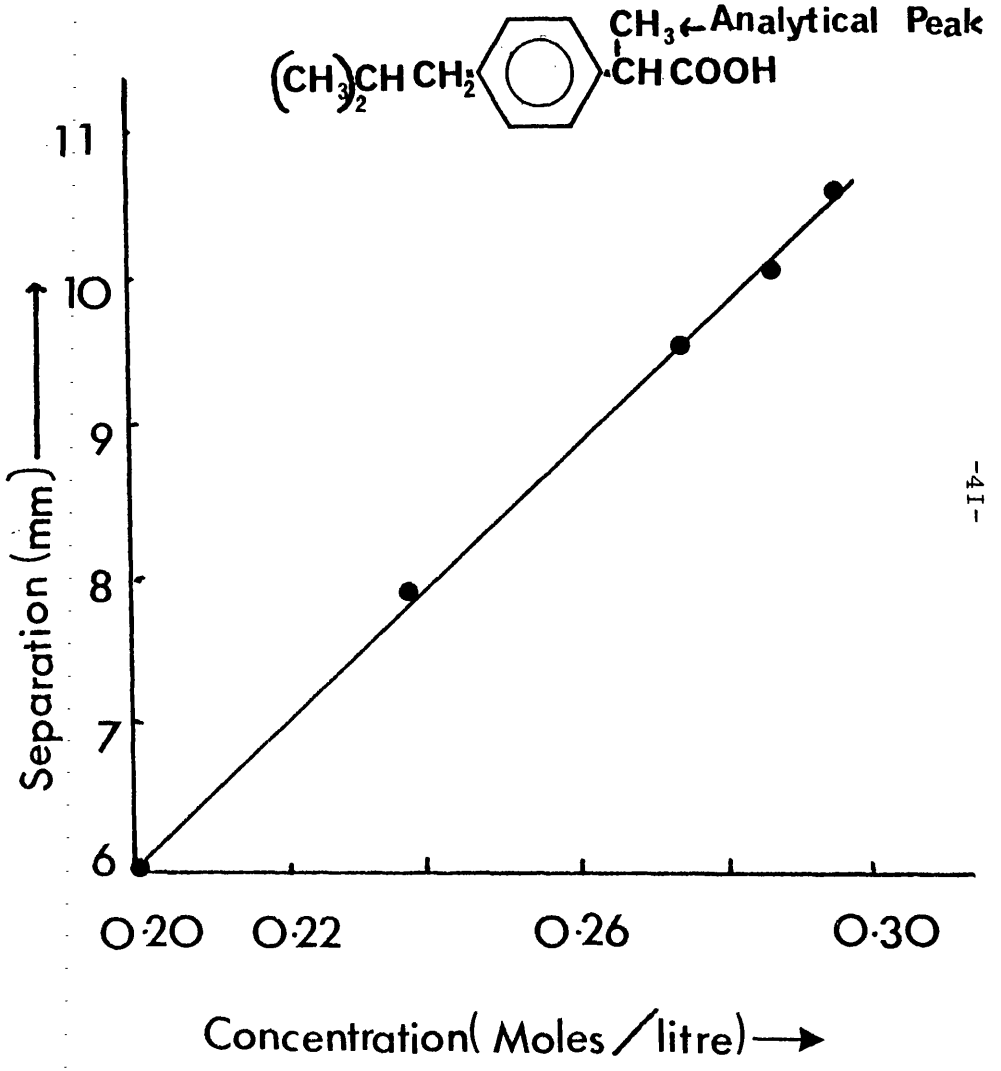
(1) There must be a constant reagent to substrate molar ratio (CLSR / Sample) for all the solutions;

(2) The instrumental conditions must be constant for all the solutions. Since the method is subject to base-line noise, recording must be stable;

FIG.2.4 RELATIONSHIP BETWEEN SUBSTRATE CONCENTRATION & SEPARATION(S)

IBUPROFEN Concentration(M)	Separation(S) (mm)
0·201	6·0
0·240	7·8
0·282	10·0
0·296	10·6
0·301	11·0

Solvent. . . . . CCl_4



(3) The total concentration of the two isomers in the mixture must be the same for all the solutions for the calibration curve.

It is not necessary for all substrate concentrations to be identical in practice, provided all results are converted to a standard concentration for calibration. Figure 2.4 (page 41) illustrates the relationship between the substrate concentration and separation (S) using results obtained from Ibuprofen. There is a linear relationship between separation (S) and concentration so that once the separation (S) for one concentration is known, the separation (S) for another concentration can be calculated.

The base line technique has been subjected to a mathematical treatment which is presented in the appendix

$$\delta h = (g(z)-f(z))\delta p - \frac{\frac{1}{2}(g'(z)-f(\frac{1}{2}))^2}{\phi''(z)} (\delta p)^2$$

This mathematical expression is summarised by saying that the base line technique is based on an equation of two terms:

(1) a linear term and

(2) a quadratic term

The quadratic term may be substantial and the linearity of the graph (curve) may only hold on a small angle.

For the enantiomers (optical purity determination) (section 2.2) the base line technique gave a linear relationship in all the compounds studied. For the diastereoisomers (section 2.3) a linear relationship

was also obtained. Even though in the case of quinine/quinidine a curve (a parabola) was obtained with the ordinary plot, the log plot gave a linear relationship (figure 2:48 page 178). On the other hand, for the non-enantiomeric or non-diasteriomeric compounds (section 2.4) a curve was predominant except where the two compounds under examination had similarity in their molecular structure. For example in the case of paracetamol and benorylate a linear relationship was obtained (figure 2:53 , page 187). It appears that the linear term of the equation is predominant for compounds of similar structures such as enantiomers and diastereoisomers. For compounds of non identical structures the quadratic term becomes significant.

The base line technique for optical purity determination has been compared with other techniques such as integration, counting of squares and weighing of the paper under the peaks. The results obtained using ibuprofen are given in Table 2.1 (page 44). The base line technique gave the best results with a % error of 0.79. Where peak separation is incomplete, the base line technique gives the most acceptable results.

For very close signals, scale expansion can prove useful. The base line technique has been compared at three different scales on the Perkin Elmer R12B spectrometer. The comparison was made using a mixture of benorylate and paracetamol in $CDCl_3$/DMSO(d_6). Three scales - 10 ppm, 5 ppm and 100 Hz - were compared. The nmr spectra of paracetamol/benorylate (Mole ratio 1.953) were recorded five times on each scale. The separation between the two $NCOCH_3$ signals

Table 2:1 Comparison of the base line technique with other techniques using results obtained from ibuprofen (section 2.2.6)

ACTUAL OPTICAL PURITY (%)	% Optical Purity as found by:				Order of accuracy
	Integration A	Counting of squares B	Weighing C	Base line technique D	
0.00	0.63	1.60	1.12	0.00	D>A>C>B
47.56	43.18	44.71	36.11	50.40	D>B>A>C
70.50	63.04	64.71	66.66	69.50	D>B>A>C
23.70	20.11	21.88	23.61	24.50	C>D>B>A
66.00	60.00	57.45	54.86	65.00	D>A>B>C
Av. % error	10.01	8.51	15.11	0.79	D>B>A>C

Table 2:2 Comparison of the base line technique at different scales, using results obtained from paracetamol/benorylate (section 2.4.1)

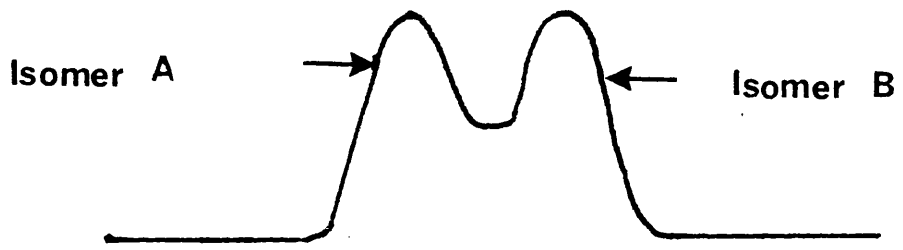
Scale	SEPARATION (S) (mm) at: 10 ppm	5 ppm	100 H_z
	23.0	30.0	32.5
	40.0	28.5	33.0
	35.0	27.2	32.8
	22.5	32.0	33.0
	21.5	26.5	32.5
Mean	30.4	28.8	32.8
Standard Deviation	8.80	2.21	0.25
Coef. of variation	28.95%	7.80%	0.77%

was measured in each case. The standard deviation and the coefficient of variation were calculated for each scale. The results are given in Table 2:2 (page 44).

The results clearly show that scale expansion gives better results than on normal scale (10 ppm). On a scale expansion the peaks are broadened and the base line F is very stable. When a large amount of lanthanide shift reagent is used, scale expansion is not necessary because in this case the peaks are very much broadened and therefore have stable base line F. The slightest separation is enough for the base line technique if a scale expansion is to be employed. The base line technique is therefore an economical method with respect to the use of CLSR.

(ii) The chemical shift difference (δv) method.

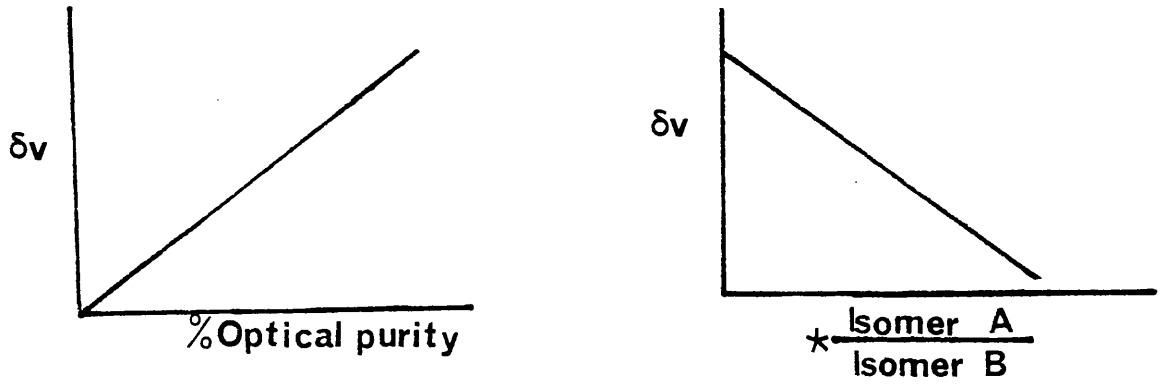
For optical purity or diastereomeric ratio determination, the chemical shift difference (δv) between the two peaks under examination from the same proton group varies proportionally with the enantiomer ratio or the diastereomeric ratio. This is illustrated below:



$\delta v \propto$ % Optical purity

or $\delta v \propto$ Diastereomeric ratio

Thus, a plot of δv against the optical purity or of δv against the ratio of the two diastereomers gives a straight line.



* where the quantity of isomer A is always smaller than isomer B.

The chemical shift difference method is applicable only when diastereoisomers are formed. For that reason the method is also applicable to enantiomers in the presence of a chiral shift reagent since diastereoisomeric complexes are formed.

Diastereomers have different chemical shifts. The chemical shift is dependent on concentration of the substrate. Therefore a diastereomer at different concentrations will exhibit different chemical shifts for a particular group of protons. Hence a mixture of two diastereomers will exhibit different chemical shift difference (δv) depending on the ratio of the two. This technique is not applicable to non diastereomers because the chemical shift difference (δv) between the two peaks under examination does not vary proportionally with the ratio of the two compounds.

This technique has been successful for all the enantiomers and diastereomers subjected to such analysis. It is interesting to note that even where the base line technique failed in the case of quinine

and quinidine for the normal plot, the chemical shift difference method still gave a linear plot. The chemical shift difference, unlike the base line technique is independent of the total concentration of the isomers. This means that there is no need to have the same concentration for all the solutions for the calibration curve. It is the molar ratio of the two isomers under examination which is important. Unlike the base line technique, the chemical shift difference is not affected by different instrumental conditions. The noise at the base line has no effect on the results.

(iii) The peak height difference (δh) method

It has been observed that if the difference in height of the two peaks belonging to the same proton group of both isomers ((+) and (-)) is plotted against the optical purity, a linear relationship is obtained. Though the idea of using a calibration curve is new, the theory behind the method is well known . Optical purity is given by

$$\frac{l-d}{l+d}$$

where 'l' and 'd' are the heights of the resonance peaks belonging to the 'l' and 'd' isomers of the same chemical group, and $l > d$. This means that a plot of (l-d) against the optical purity must be a straight line.

The direct use of peak height as a means of nmr quantification is not entirely satisfactory and this will be elaborated in part 3 of this book. Moreover, the peak height difference is not zero for many racemic compounds (see section 2.2). Therefore the use of the above formula for enantiomer quantification has serious limitations.

The peak height difference, like the base line separation (S), is dependent on the total concentration of the substrate, and the instrumental conditions.

All the three techniques - the base line technique, the chemical shift difference method and the peak height difference method - had a correlation coefficient greater than 0.97 in all the compounds analysed. The accuracy and precision of the three techniques have been compared. A typical result obtained is represented in table 2:3 (page 49). Although all the three techniques show acceptable accuracy the chemical shift difference method appears to be the most accurate, and with the least standard deviation. The main disadvantage of the techniques is the fact that the pure isomers or at least one pure isomer in addition to the racemate must be available for a calibration curve.

Derivatisation of compounds

Derivatisation of the compounds was sometimes necessary in order to obtain the optical purity. Derivatisation is essential where an appropriate signal is absent in the compound, the compound under examination possesses many co-ordinating groups and therefore gives broad and featureless peaks, or the compound is insoluble in any of the common solvents employed for the analysis (see table 2:4, page 51).

(i) Compounds with no appropriate signal

Two of the compounds - atropine/hyosyamine (2:21) (section 2.2.10) and propranolol (2:5) (section 2.2.3) - fell within this category. For atropine/hyosyamine, the N-CH_3 signal appeared to

Table 2:3 Comparison of the base line technique, δv method and δh method using results obtained from Naproxen (section 2.2.7).

ACTUAL OPTICAL PURITY (%)	% optical purity obtained by: Base line technique	δv method	δh method
0.0	0.2	0.0	0.0
25.6	24.7	25.3	25.0
50.5	49.0	50.7	51.2
70.3	70.8	70.0	70.6
Av. % error	0.75	0.20	0.53
Av. Standard Deviation	2.5	0.1	2.2

be suitable at the initial stages. However, with the addition of the shift reagent (see section 2.2.10) the CH_2 signals merged with the CH_3 peaks. It was therefore necessary to use a derivative. The most appropriate derivative was the tropic acid methyl ester.

In the case of propranolol, the spectrum showed signal overlap (with the exception of the $C(CH_3)_2$ signal) Addition of a shift reagent did not result in resonance separation. The $C(CH_3)_2$ signal could not be used in view of non-equivalent of the methyl signals which was revealed with addition of a shift reagent (see section 2.2.3). The *N*-acetyl derivative proved successful.

(ii) Compounds possessing many co-ordinating groups.

Compounds in this category present two problems, one of which is solubility and the other broad bands. Three of the compounds studied - salbutamol, dopa and rimiterol- fell into this category and therefore required derivatisation. Appropriate derivatives would increase their solubility in organic solvents like $CDCl_3$, CCl_4 and C_6D_6, and also shield co-ordination sites by steric hindrance. Trimethylsilyl (TMS) derivatives were found to be the derivatives of choice. *N,N*,-bis-trimethylsilyltrifluoroacetamide (BSTFA) (2:1) was the silylating agent employed. WIth (2:1), trifluoroacetamide is formed in addition to the silylated product, but its volatility facilitated its removal.

$$\left[\cdot\ (CH_3)_3Si\right]_2 \text{ —— } N - \overset{\overset{O}{\|}}{C} - CF_3$$

(2:1)

Table 2:4 Samples for the optical purity determination and their method of analysis

Sample	Compound No.	Method of Analysis	Reasons for Derivatization
Ephedrine/pseudo-ephedrine	2:4 2:5	Direct application of CLSR	-
Propranolol	2:6	Derivatization: *N*-acetyl propranolol	No appropriate signal for the analysis.
Salbutamol	2:8	Derivatization: $(OTMS)_3$ Salbutamol	Compound containing many co-ordinating groups and Insolubility
Tetramisole	2:11	Direct application of CLSR	-
Ibuprofen	2:13	Direct application of CLSR	-
Naproxen	2:16	Direct application of CLSR	-
Ketoprofen	2:17	Derivatization: the methyl ester derivative	No appropriate signal for analysis
Fenoprofen	2:19	Derivatization: the methyl ester derivative	Solubility problem. The calcium salt was supplied.
Atropine/ hyoscyamine	2:21	Derivatization: tropic acid methyl ester	No appropriate signal for analysis
Dopa	2:27	Derivatization: Silyl derivative of the methyl ester	Many co-ordinating groups; no appropriate signal.

(iii) Compounds with solubility problems other than those in (ii)

Fenoprofen (2:19) is an example in this category. Fenoprofen is formulated as the calcium salt. This salt is not soluble in any of the normal organic solvents used for the optical purity determination. It is also difficult to obtain fenoprofen(2:19) from this salt by ordinary solvent extraction. The solution to the problem was the use of a derivative. In this case a methyl ester was formed directly from the salt and then extracted into chloroform.

The choice of a derivative obviously depends on the parent compound itself but to make the analysis meaningful the derivative must be easy to prepare. The time involved must be short so that many determinations can be made in a short time.

There are many problems associated with the preparation of derivatives, the most serious of which is the possibility of racemisation of the pure isomer. One of the compounds - hyoscyamine - presented this problem. Alkaline hydrolysis followed by extraction with chloroform resulted in the formation of racemic tropic acid instead of the l-form (see section 2.2.10).

Experimental difficulties and limitations of the method

One problem with the optical purity determination by use of CLSR is the choice of solvent. Three solvents - $CDCl_3$, CCl_4 and C_6D_6 - were used throughout the analysis. Though there was no one solvent which could claim entire superiority over the others, CCl_4 was the best solvent for most of the compounds in view of the large differential shift obtained. It is closely followed by C_6D_6.

[Figures 2:13 (page 89), 2:17 (page 98), 2:36 (page 148) and 2:39 (page 157) show the effect of solvents on LIS and ΔLIS of various compounds]. The least useful of the three solvents - $CDCl_3$ - for differential shifts, was the best solvent with regard to solubility of the compounds studied, and CCl_4 the worst. In some cases, a combination of solvents proved useful.

In general the best choice of resonance signal for the analysis is one

(i) with high sensitivity;

(ii) which is a singlet;

(iii) free from interference of other resonance peaks;

(iv) close to the chiral centre and

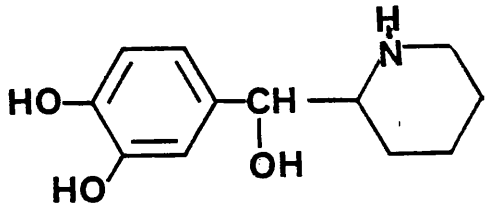
(v) distant from the co-ordination centre.

The last requirement (v) prevents excessive broadening.

A preliminary study of the compound under examination with non-chiral shift reagent was the practice adopted throughout the experiments. This was to find out the behaviour of the compound in the presence of LSR and hence to avoid the use of the more expensive CLSR on less promising compounds.

The use of chiral lanthanide shift reagents for enantiomer quantification is not always successful. Two out of the group of thirteen compounds studied could not be successfully quantified using CLSR. These were Rimiterol (3,4-dihydroxyphenyl-(2-piperidyl) carbinol (2:2) and Camphor (2:3).

Rimiterol is a sympathomimetic bronchodilator. It is normally supplied as the hydrobromide salt. As with salbutamol (section 2.2.4).,

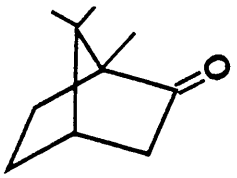


(2:2)

rimiterol presented solubility problems. Several spectra of rimiterol hydrobromide in many solvents were recorded but a broad and featureless spectrum was obtained in each case due to viscosity of the solutions. The solvents employed included: D_2O, DMSO(d_6), $CDCl_3$/DMSO(d_6) and C_6D_6/DMSO(d_6). In view of the broad resonances, complete assignment of the peaks was impossible. When working on rimiterol HBr, Sankey and Whiting[108] obtained the nmr spectrum of a sample in D_2O at 95°C. To obtain a good nmr spectrum of rimiterol HBr, a high temperature probe is required. Obviously 37°C is too low a temperature to obtain a good spectrum of rimiterol HBr. Attempts to use rimiterol itself also failed.

The failure of the rimiterol optical purity determination was due to viscosity of its solutions for nmr spectra. Paramagnetic material(s) in viscous solutions result(s) in broadened peaks. A variable temperature probe might be the solution to the problem.

For the camphor optical purity determination, the failure was due to lack of appropriate shift reagents since the shift reagents employed were camphor derivatives .



(2:3)

Despite some limitations, the use of CLSRs for optical purity determination would seem to have some merit. It is accurate, fast and easy to use. For direct determination the method involves only four steps.

(i) Identification of resonance signals of the compound under examination.

(ii) Preliminary study with non chiral shift reagents.

(iii) Determination of ΔLIS with CLSRs.

(iv) THe optical purity determination.

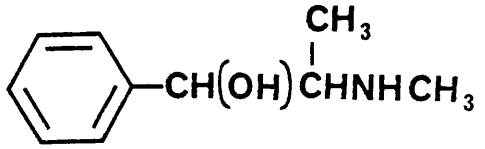
CLSRs offer a quick method of checking the enantiomeric ratio of compounds. It is a highly sensitive method in the sense that it requires only a few milligrams of the sample for the analysis (unlike polarimetry). Where complete separation of the analytical peaks is obtained, as in the case of tetramisole (section 2.2.5), the analysis takes only a few minutes.

For direct determination of the enantiomeric ratio, the method is limited to compounds with few co-ordinating groups (not more than 3). However, the method could be adopted for any compound through derivatisation and the use of appropriate shift reagents and under the right instrumental conditions. Where there is complete separation of the two resonance peaks belonging to the two isomers under

examination, and integration is used for the optical purity determination, the % error is < 0.4.

2.2 ENANTIOMERS

2.2.1 EPHEDRINE



(2:4)

Ephedrine (2:4) is an alkaloid obtained from species of *Ephedra*, or prepared synthetically. It is a sympathomimetic amine with a more prolonged, though less potent, action than adrenaline. Since the discovery of the Ephedrines in 1887[109] numerous articles have been published with regard to their structure[110, 111], conformational analysis[112, 113], studies on their configuration[114] and their properties, including biological activity. Though much work has been done on Ephedrines by the use of nmr, none includes the quantitative analysis by this technique. In this report an account is given on the potential use of nmr for the direct determination of their optical purities using chiral lanthanide shift reagents.

Before the optical purity determination, the Lanthanide induced shifts (LIS) of the various proton groups in Ephedrine were determined using $Eu(fod)_3$ and $Pr(fod)_3$. The results are shown in figure 2.5 (page 63) and in tables 2.5A and B (page 58). The differential shifts (ΔLIS or $\Delta\Delta\nu$) were also determined using $Eu(facam)_3$ and $Pr(facam)_3$. The results for this determination is shown in figure 2.6 (page 67) and in tables 2:5C and D.

The optical purity was determined using $Pr(facam)_3$ in C_6D_6 and

Table 2:5A LIS of the various proton groups in ephedrine in the presence of $Pr(fod)_3$ in benzene

[Ephedrine] = 0.45M

MOLAR RATIO $\frac{Pr(fod)_3}{ephedrine}$	LIS in ppm							
	C-CH_3		N-CH_3		CH-N		O-CH	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν
0.00	0.74	0.00	2.10	0.00	2.54	0.00	4.71	0.00
0.060	0.04	0.70	0.84	1.26	1.68	0.86	3.85	0.86
0.093	-0.27	1.01	0.74	1.36	1.23	1.31	3.35	1.36
0.136	-0.64	1.38	0.67	1.43	0.67	1.87	2.78	1.93

Table 2:5B LIS of the various proton groups of Ephedrine in C_6D_6 using $Eu(fod)_3$

[Ephedrine] = 0.46M

MOLAR RATIO $\frac{Eu(fod)_3}{Ephedrine}$	C-CH_3		N-CH_3		O-CH		*Aromatic protons	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν
0.00	0.67	0.00	2.05	0.00	4.68	0.00	7.15	0.00
0.045	0.90	0.23	1.97	-0.08	4.57	-0.11	7.00	-0.15
0.096	1.02	0.35	1.88	-0.17	4.52	-0.16	6.85	-0.30
0.136	1.11	0.44	1.81	-0.24	4.47	-0.21	6.75	-0.40
0.182	1.20	0.53	1.72	-0.33	4.46	-0.22	6.63	-0.52
0.240	1.39	0.72	1.56	-0.49	4.47	-0.21	6.47	-0.68
0.292	1.53	0.86	1.40	-0.60	4.75	+0.07	6.30	-0.85

*Average results for the aromatic protons

The *CH*N resonances were broad and featureless

-ve values indicate upfield shift.

Table 2:5C LIS and $\Delta\Delta\nu$ of the C-CH_3 signals of d and l-ephedrins in benzene using Pr(facam)$_3$

[dl-Ephedrine] = 0.39M

MOLAR RATIO	l-isomer		d-isomer		$\Delta\Delta\nu$ (ppm)
	δ	$\Delta\nu$	δ	$\Delta\nu$	
0.00	0.74	0.00	0.74	0.00	0.00
0.076	0.05	0.69	0.18	0.56	0.13
0.119	-0.34	1.08	-0.16	0.90	0.18
0.153	-0.59	1.33	-0.35	1.09	0.24
0.182	-0.86	1.60	-0.57	1.31	0.29
0.212	-1.19	1.93	-0.86	1.60	0.33

Table 2:5D LIS and $\Delta\Delta\nu$ of the N-CH_3 signals of d and l-ephedrines in benzene using Eu(facam)$_3$

[dl-Ephedrine] = 0.40 M

MOLAR RATIO Eu(facam)$_3$ / dl-ephedrine	l-isomer		d-isomer		$\Delta\Delta\nu$ (ppm)
	δ	$\Delta\nu$	δ	$\Delta\nu$	
0.00	2.10	0.00	2.10	0.00	0.00
0.065	1.91	0.19	1.98	0.12	0.07
0.090	1.84	0.26	1.93	0.17	0.09
0.108	1.77	0.33	1.89	0.21	0.12
0.120	1.75	0.35	1.88	0.22	0.13
0.172	1.64	0.46	1.81	0.29	0.17

Table 2:6A Optical Purity determination of Ephedrine

Shift reagent: $Pr(facam)_3$

Solvent: C_6D_6 (0.7 mls) [Total Ephedrine] = 0.30 M

*Molar Ratio: 0.216 ± 0.004

Analytical peak: C-CH_3 signal at -1.0 to -1.5 ppm

Optical purity (OP; %)	Separation (S; mm)	$1 - \frac{OP}{100}$	Peak height difference (δh; mm)	Chemical shift difference (δv) (ppm)
0.0	4.70	1.00	-0.60	0.30
25.6	3.80	0.74	5.34	0.46
47.3	2.71	0.53	11.76	0.60
76.0	1.48	0.24	20.69	0.77

Table 2:6B Optical Purity determination of Ephedrine using peak height

Shift reagent: $Eu(facam)_3$

Solvent: benzene [Total Ephedrine] = 0.30 M

*Molar Ratio: 0.11

Analytical Resonance peak - N-CH_3 at δ1.7 to 1.9.

Optical purity found (O; %)	Actual optical purity (A; %)	$\frac{O}{A}$ x 100(%)
0.00	0.00	100
10.13	10.20	99.31
21.77	21.95	99.2
28.35	28.21	100.5
35.00	35.05	99.9
36.74	37.51	98.0
42.42	41.21	103.0
48.04	46.09	104.2
53.10	51.79	102.5
	Mean	100.7
	Standard deviation	2.06

* Molar Ratio = $\frac{\text{Reagent}}{\text{Ephedrine}}$

Table 2:7A Effect of dilution with benzene on the LIS of ephedrine at $Eu(fod)_3$/ephedrine mole ratio of 0.159

Vol. of C_6D_6 added (ml)	C-CH_3		N-CH_3		O-CH	
	δ	Δv	δ	Δv	δ	Δv
Original	0.91	0.00	1.68	0.00	4.22	0.00
0.1	0.99	0.08	1.74	0.06	4.33	0.11
0.2	1.07	0.16	1.81	0.13	4.46	0.24
0.3	1.14	0.23	1.87	0.19	4.58	0.36
0.4	1.22	0.31	1.92	0.24	4.69	0.47
0.5	1.29	0.38	1.97	0.29	4.76	0.54

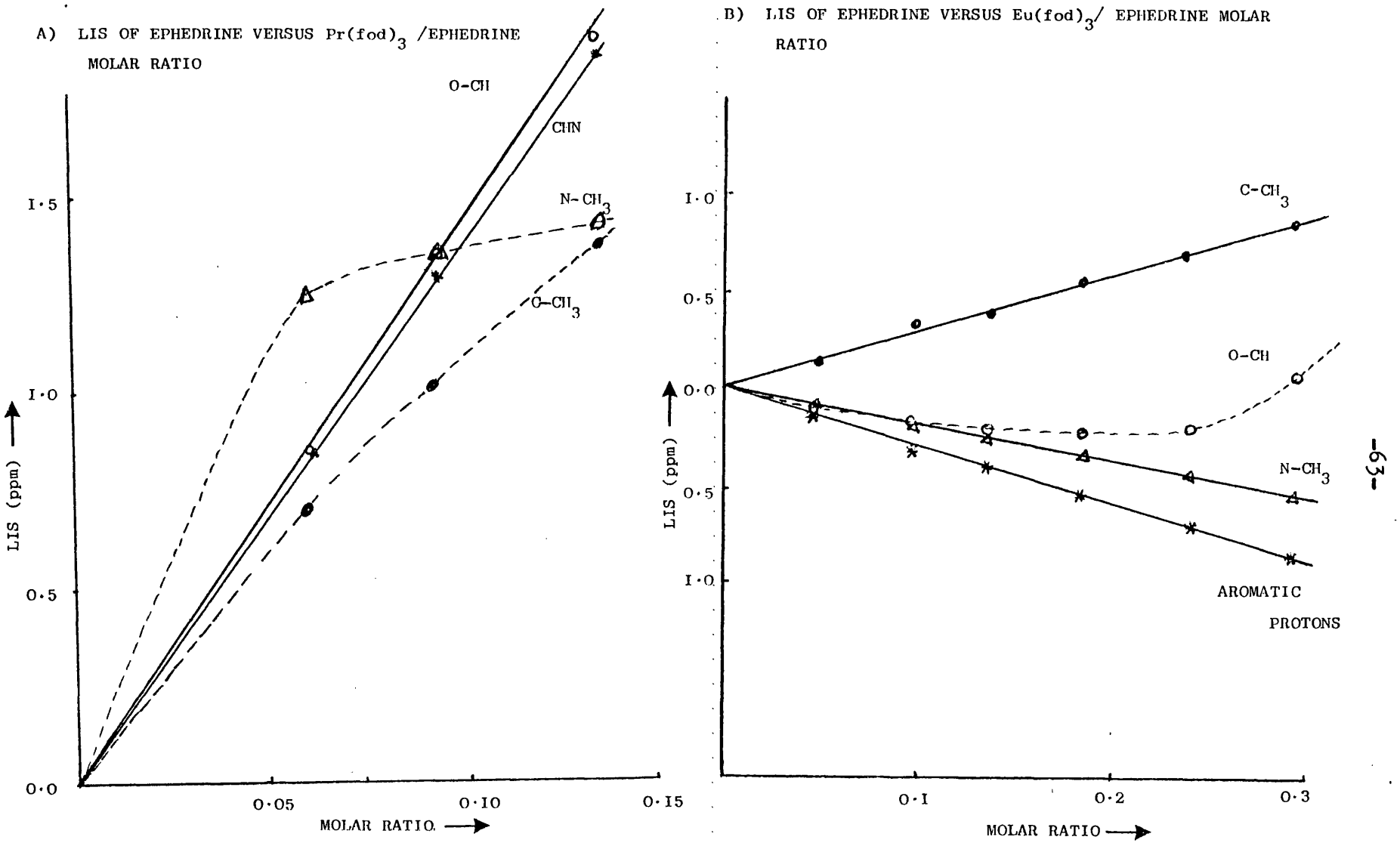
Table 2:7B Effect of dilution with benzene on the LIS of ephedrine at $Eu(fod)_3$/ephedrine molar ratio of 0.212

Vol. of C_6D_6 added (ml)	C-CH_3		N-CH_3		O-CH	
	δ	Δ_v	δ	Δ_v	δ	Δ_v
Original	1.13	0.00	1.75	0.00	4.37	0.00
0.1	1.23	0.10	1.79	0.04	4.52	0.15
0.2	1.34	0.21	1.89	0.14	4.68	0.31
0.3	1.42	0.29	1.92	0.17	4.80	0.43
0.4	1.47	0.34	1.95	0.20	4.89	0.52
0.5	1.52	0.39	1.97	0.22	4.97	0.60

Table 2:7C Effect of dilution of ephedrine in C_6D_6 at a molar ratio of 0.106 using $Eu(fod)_3$

Vol. of C_6D_6 added (ml)	C-*CH*$_3$		N-C*H*$_3$		O-*CH*	
	δ	Δν	δ	Δν	δ	Δν
Original	0.90	0.00	1.84	0.00	4.41	0.00
0.1	1.00	0.10	1.93	0.09	4.53	0.12
0.2	1.04	0.14	1.96	0.12	4.61	0.20
0.3	1.05	0.15	1.98	0.14	4.63	0.22
0.4	1.05	0.15	1.98	0.14	4.67	0.25

FIG. 2:5 LIS of Ephedrine in C_6D_6



by employing the base line technique, δv method and δh methods. The C-*CH*$_3$ was used as the analytical peak. The results are given in figure 2.7 (page 68) and in table 2:6A (page 60). Direct peak height measurements were also used for the optical purity determination by the use of Eu(facam)$_3$. The results are given in table 2:6B (page 60).

2.2.1.1 Results and discussion

Lanthanide induced shifts (LIS):

The problem encountered initially with the determination of LIS was the choice of suitable solvent. The use of solvents containing hetero atoms such as oxygen, nitrogen and sulphur were precluded in view of competition between the ephedrines and the solvents for the shift reagent. The most readily available solvents - $CDCl_3$ and CCl_4 - which are most often used with lanthanide shift reagents proved to be unsuitable. The ephedrines dissolved with difficulty in $CDCl_3$ and CCl_4 at room temperature, and in many cases crystallized out when inserted in the probe. Brownlee[115] has reported that ephedrine base dissolved in $CHCl_3$ or CCl_4 forms its hydrochloride.

Benzene proved to be the most suitable. With Pr(fod)$_3$ as the shift reagent and benzene (d_6) as the solvent, all the various proton groups gave upfield shifts which is expected from praseodymium. (The results are shown in figure 2:5 and in table 2:5). The *OH* and *NH* moved together and the extent of LIS for the various groups indicated equal co-ordination at both *O* and *N* atoms. With Eu(fod)$_3$ in benzene (d_6) the various groups (with the exception of *OH*, *NH* and C-CH_3) showed upfield shift at Eu(fod)$_3$/Ephedrine molar ratio

below 0.3. Above this ratio, the $\overset{O}{\overset{|}{C}}H$ signal gave a shift reversal. At low molar ratio of reagent to substrate, the site of co-ordination appears to be both *N* and *O* . At higher molar ratios, the co-ordination is stronger at *O* than at the *N*.

LIS and ΔLIS with chiral shift reagents:

The results for the ΔLIS are given in figure 2:6 and in tables 2:5C and 2:5D. With $Pr(facam)_3$ as the shift reagent in benzene, the $N\text{-}CH_3$ and the $C\text{-}CH_3$ gave differential shifts ($\Delta\Delta\nu$) for the l and d isomers. However, the signals were too broad particularly for the $N\text{-}CH_3$ signal. It appears that with $Pr(facam)_3$ in benzene, the co-ordination is stronger at *N* than at *O*, since the groups attached to the *N* (i.e. $N\text{-}CH_3$ and *CH*N) gave the largest LIS. Moreover, they gave the most broadened peaks (line broadening $\alpha\ 1/r^6$)[61,64], at the concentrations of the reagent used.

With $Eu(facam)_3$ in benzene at the concentrations used, the $N\text{-}CH_3$ gave two sharp singlets (figure 2:1 page 36). The $C\text{-}CH_3$ also showed some separation. Of the two groups the $N\text{-}CH_3$ showed the greater promise for analytical purposes, because the resonance peaks of the d and l isomers were well separated even at a $Eu(facam)_3$/dl-ephedrine molar ratio of 0.1. Higher concentration of the reagent is likely to give well separated peaks more suited to analysis but the presence of irrelevant resonance peaks (from the reagent) close to the $N\text{-}CH_3$ peaks, and the possibility of the $N\text{-}CH_3$ and $C\text{-}CH_3$ peak overlap at higher reagent concentrations, indicates a reagent to substrate mole ratio of about 0.11 to be the most suitable for optical purity determination.

Optical purity determination:

For the peak height measurements the optical purity was calculated from the following formula:

$$\text{optical purity} = \frac{H_2 - H_1}{H_1 + H_2} \times 100$$

where H_2 and H_1 are the heights of the N-C*H*$_3$ resonance peaks of the l and d isomers. The subscripts (2 and 1) indicate the higher and lower peaks respectively. The results were satisfactory up to optical purity of about 50% (see table 2:6B). Above that, unsatisfactory results were obtained due to broadened peaks.

With the new techniques - (base line, δv and δh) - a linear relationship was obtained when the optical purity or 1 - % optical purity/100 was plotted against the appropriate parameter. The results are shown in figure 2:7 and in table 2:6A.

Effect of dilution on the LIS:

During the course of the experiments it was observed that at a fixed reagent-substrate mole ratio, incremental addition of the solvent caused a downfield shift proportional to the amount of solvent added. This effect was independent of the nature of the reagent or solvent used but depended on the initial concentration of the reagent. The results are given in tables 2:7A to C. (page 61) and in figure 2:8 (page 69) . This dilution effect has already been observed by some workers[116] but no explanation had been given for it. Tomic *et al.*[116] observed the effect on cyclopropylmethanols with Pr(DPM)$_3$ in CCl_4, and CS_2, when dilution with the solvent gave downfield shifts. Since praseodymium chelates normally give upfield shifts, the downfield shift brought as a result of dilution might have been assumed to be

FIG. 2 : 6 $\Delta\Delta^{v}$ /Chemical shift VRS Molar ratio of CLSR / Ephedrine

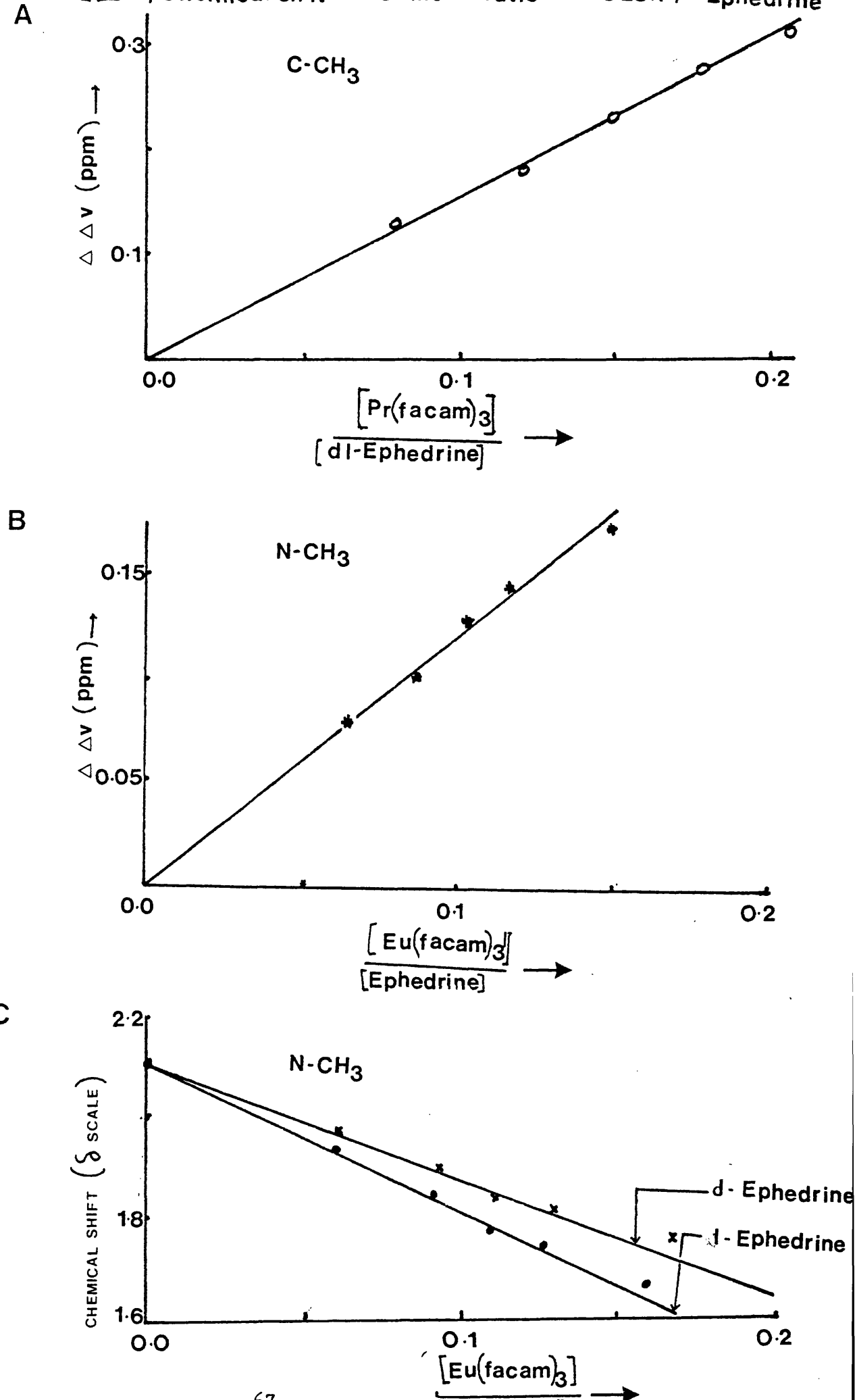
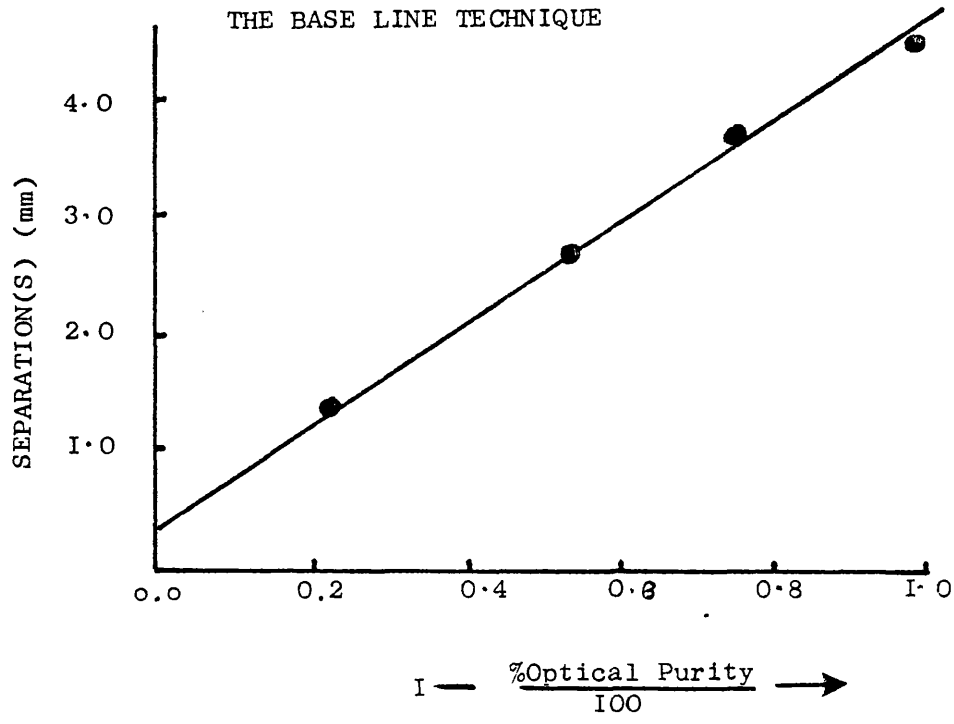
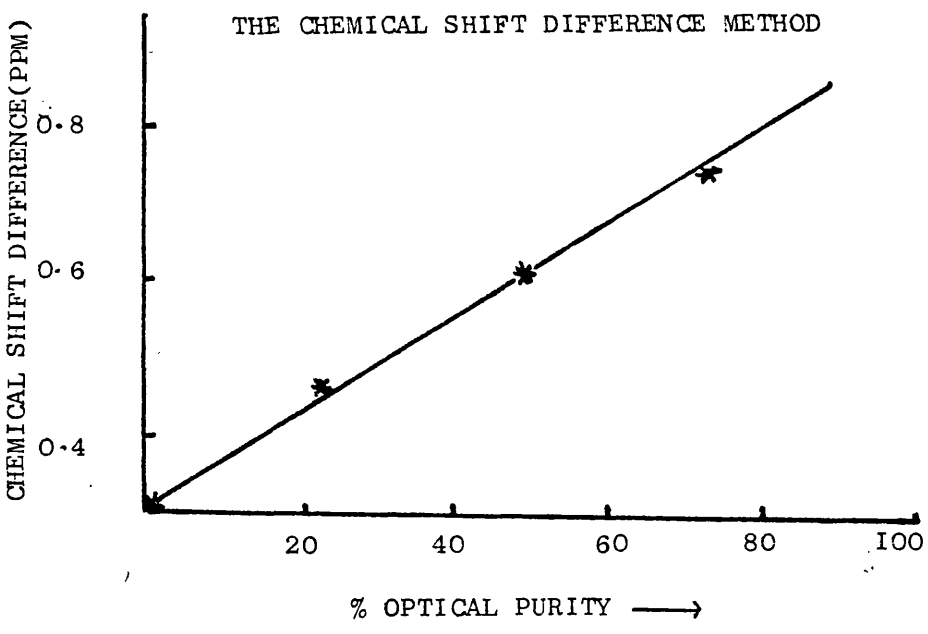


FIG. 2:7 THE OPTICAL PURITY DETERMINATION OF EPHEDRINE.





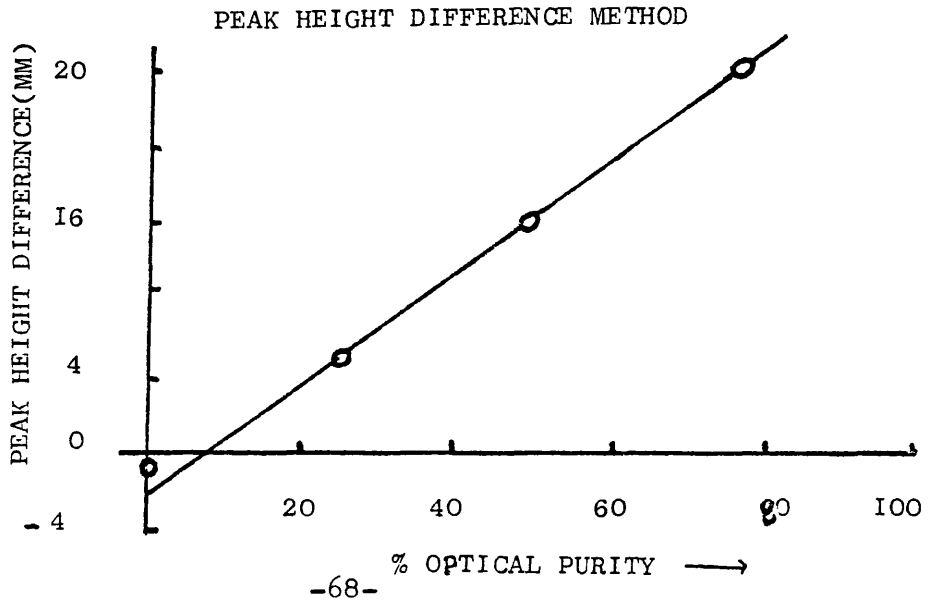
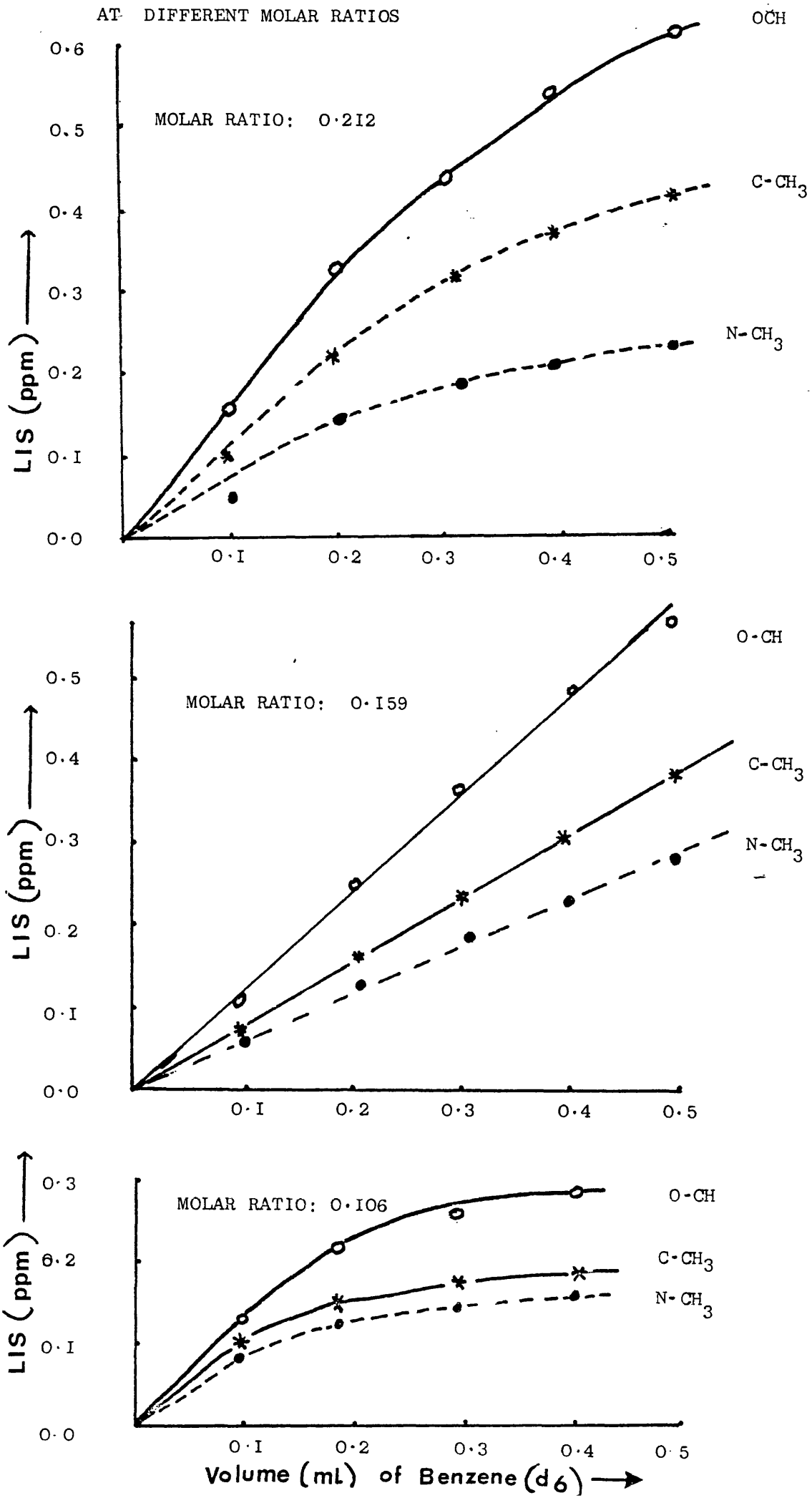
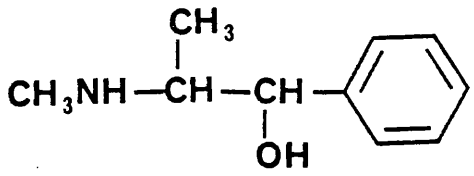


FIG.2;8 EFFECT OF DILUTION ON THE LIS OF EPHEDRINE AT DIFFERENT MOLAR RATIOS



a reversal of shift. In the present experiments a europium chelate $Eu(fod)_3$ was used on dl-ephedrine in benzene (d_6) and all proton groups in the molecule gave downfield shifts with dilution irrespective of their initial direction of shift. This effect, therefore, cannot be regarded as shift reversal. Irrelevant resonance peaks such as solvent peaks and peaks due to the reagent all moved downfield with dilution. The effect is greater at higher molar ratios.

2.2.2 PSEUDO EPHEDRINE



(2:5)

Pseudo ephedrine (2:5) is a diastereoisomer of ephedrine. It is a bronchodilator and peripheral vasoconstrictor. It is said to have a less vasopressor action than ephedrine and to cause little cerebral stimulation. It is used as a nasal and bronchial decongestant, particularly in bronchial asthma[117].

Like ephedrine, the optical purity of pseudo ephedrine has been determined by application of the base line technique, chemical shift difference and peak height difference methods. $Pr(facam)_3$ was the CLSR and C-CH_3 was used as the analytical peak. The results are

Table 2:8A LIS of the various groups in pseudo ephedrine in C_6D_6 using $Eu(fod)_3$

[ψ-ephedrine] = 0.31M

MOLAR RATIO $\frac{Eu(fod)_3}{\psi\text{-ephedrine}}$	C-C*H*$_3$		N-C*H*$_3$		O C*H*		Aromatic protons	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν
0.00	0.66	0.00	2.00	0.00	4.03	0.00	7.17	0.00
0.072	0.67	0.01	0.96	-1.04	4.20	0.17	7.00	-0.17
0.135	0.73	0.07	0.81	-1.19	4.37	0.34	6.83	-0.34
0.205	0.76	0.10	0.62	-1.38	4.62	0.59	6.64	-0.53
0.275	0.80	0.14	0.40	-1.60	5.02	0.99	6.43	-0.74

-ve values indicate upfield shift.

N*H*, O*H* and C*H* gave downfield shift.

Table 2:8B LIS and ΔLIS of dl-ψ-ephedrine in benzene (d_6) using $Eu(facam)_3$

[Pseudo ephedrine] = 0.30m

MOLAR RATIO $\frac{Eu(facam)_3}{dl\text{-}\psi\text{-ephedrine}}$	C-CH_3 d		C-CH_3 l			N-CH_3		* O‖CH		Aromatic protons	
	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	δ	Δν
0.00	0.66	0.00	0.66	0.00	0.00	1.99	0.00	4.04	0.00	7.13	0.00
0.062	0.67	0.01	0.64	-0.02	0.03	2.20	0.21	4.10	0.06	7.03	-0.10
0.138	0.68	0.02	0.62	-0.04	0.06	2.46	0.47	4.18	0.14	6.92	-0.21
0.199	0.68	0.02	0.58	-0.08	0.10	2.57	0.58	4.18	0.14	6.76	-0.37
0.265	0.65	-0.01	0.54	-0.12	0.11	2.39	0.40	4.13	0.09	6.58	-0.55
0.403	0.53	-0.13	0.42	-0.24	0.11	2.04	0.05	4.09	0.05	6.24	-0.89
0.550	+		+		+	1.41	-0.58	+		5.71	-1.42
0.715	-0.36	-1.02	-0.36	-1.02	0.00	0.90	-1.09	+		5.16	-1.97

* Average results for the resonance peaks.

Table 2:8C LIS and ΔLIS of dl-ψ-ephedrine in $CDCl_3$ using $Eu(facam)_3$

[Pseudo ephedrine] = 0.30M

MOLAR RATIO $\frac{Eu(facam)_3}{dl\text{-}\psi\text{-ephedrine}}$	d $C\text{-}CH_3$					$N\text{-}CH_3$		$\overset{O}{\underset{CH}{\vert}}$		Aromatic protons	
	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	δ	Δν
0.00	0.93	0.00	0.93	0.0	0.00	2.43	0.00	4.26	0.00	7.32	0.00
0.068	0.88	-0.05	0.82	-0.11	0.06	2.60	0.17	4.20	-0.06	7.06	-0.26
0.120	0.89	-0.04	0.80	-0.13	0.09	2.82	0.39	4.38	0.12	6.94	-0.38
0.185	0.84	-0.09	0.73	-0.20	0.11	3.02	0.59	4.51	0.25	6.69	-0.63
0.229	0.78	-0.15	0.67	-0.26	0.11	3.28	0.85	4.89	0.72	6.32	-1.00

Table 2:8D LIS and ΔLIS of dl-ψ-ephedrine in C_6D_6 using $Pr(facam)_3$

[Pseudo ephedrine] = 0.30M

MOLAR RATIO	C-CH_3					N-CH_3					Aromatic	
$Pr(facam)_3$	d		l			d		l			protons	
dl-ψ-ephedrine	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	ΔΔν	δ	Δν
0.00	0.67	0.00	0.67	0.0	0.00	2.01	0.0	2.01	0.0	0.00	7.16	0.00
0.035	0.29	-0.38	0.36	-0.31	0.07	1.16	-0.85	1.06	-0.95	0.10	7.08	-0.08
0.067	0.00	-0.67	0.11	-0.56	0.11	0.54	-1.47	0.35	-1.66	0.19	7.05	-0.11
0.182	-1.21	-1.88	-0.97	-1.64	0.24	-2.18	-4.19	-2.52	-4.53	0.34	6.78	-0.38
0.230	+		+			+		+			6.76	-0.40

+ - Resonance peaks broad and featureless.

Table 2:8E LIS and ΔLIS of dl-ψ-ephedrine in $CDCl_3$ using $Pr(facam)_3$

[Pseudo-ephedrine] = 0.30M

MOLAR RATIO	C-CH_3					N-CH_3					Aromatic protons	
$\frac{Pr(facam)_3}{dl\text{-}\ \text{-Ephedrine}}$	d		l			d		l				
	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	ΔΔν	δ	Δν
0.00	0.91	0.00	0.91	0.0	0.00	2.39	0.00	2.39	0.0	0.0	7.31	0.0
0.060	0.38	-0.53	0.28	-0.63	0.10	1.10	-1.29	1.00	-1.39	0.10	7.13	-0.18
0.114	*		*			*		*			6.95	-0.36
0.166	-0.80	-1.71	-1.04	-1.95	0.24	-1.69	-4.08	-1.93	-4.32	0.24	6.74	-0.57
0.221	-1.52	-2.43	-1.80	-2.71	0.28	-3.27	-5.66	-3.53	-5.92	0.26	6.53	-0.78
0.267	-2.21	-3.12	-2.56	-3.47	0.35	⊕		⊕			6.17	-1.14

* Resonance peaks superimposed

⊕ Resonance peaks superimposed on irrelevant peaks

Table 2:9A Determination of Optical Purity of ψ-Ephedrine by the application of the Base Line Technique, Chemical Shift difference and Peak height difference methods.

% OPTICAL PURITY (OP)	$1 - \frac{OP}{100}$	Separation(S) (mm)	Chemical Shift difference (δv) (mm)	Peak Height difference (d-l) (mm)
0.0	1.00	14.5	0.28	0.0
20.0	0.80	11.6	0.32	6.0
55.0	0.45	6.5	0.38	15.0
70.0	0.30	4.5	0.41	20.5
100.0	0.00	-	-	29.0

Solvent: $C_6D_6/CDCl_3$ (1:1)

Molar Ratio: Reagent / ψ-Ephedrine = 0.221

Reagent: $Pr(facam)_3$ [ψ-Ephedrine] ≒ 0.30M

Analytical Peak - C-CH_3

Resonance position: d = -δ1.52

l ≒ -δ1.80

Table 2:9B Optical Purity determination of ψ-Ephedrine by peak height measurement

Molar Ratio: Reagent / ψ-Ephedrine 0.075

Reagent: $Pr(facam)_3$; [Pseudo ephedrine] = 0.30M

Solvent: $CDCl_3/C_6D_6$ (1:1)

Analytical peak: N-CH_3

Actual % Optical Purity (A)	% Optical Purity obtained (O)	O/A x 100%	% Error
0.0	1.20	-	-
9.70	10.98	113.20	13.2
14.44	12.56	86.98	13.0
23.81	20.67	86.81	13.2
28.12	25.12	89.33	10.7
33.99	Unsuccessful	-	
50.00	Unsuccessful	-	

Table 2:10 Effect of dilution with benzene on the LIS of the various groups of ψ-Ephedrine at a fixed $Eu(fod)_3$/ψ-Ephedrine mole ratio of 0.275

Volume of benzene added (ml)	$C\dot{-}CH_3$		$N-CH_3$		O CH		Aromatic protons	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν
Original	0.80	0.0	0.40	0.00	5.02	0.0	6.43	0.0
0.05	0.97	0.17	0.56	0.16	5.33	0.31	6.62	0.19
0.07	1.00	0.20	0.61	0.21	5.34	0.32	6.64	0.21
0.12	1.10	0.30	0.72	0.32	5.48	0.46	6.73	0.30
0.22	1.18	0.38	0.81	0.41	5.65	0.63	6.80	0.37
0.32	1.21	0.41	0.84	0.44	5.75	0.73	6.83	0.40

Initial pseudo ephedrine concentration = 0.30M

given in table 2:9B (page 77). The LIS and ΔLIS were also determined and the results are shown in tables 2:8A-E (page 71). A graph of ΔLIS is shown in figure 2:9 (page 80). The lanthanide shift reagents employed for the analysis were $Eu(fod)_3$, $Pr(fod)_3$, $Eu(facam)_3$ and $Pr(facam)_3$.

2.2.2.1 Results and Discussion

LIS and ΔLIS:

The LIS using the various shift reagents in C_6D_6 were slightly different from those of ephedrine. This is obviously due to the different configurations of ephedrine and pseudo-ephedrine.

With the $Eu(facam)_3$ in benzene (d_6), the C-CH_3 and O-CH signals gave differential shift. The N-CH_3 signal unlike that of ephedrine, did not separate.

It is interesting to note that pseudo ephedrine, in constrast to ephedrine, is readily soluble in $CDCl_3$. The LIS obtained in $CDCl_3$ are significantly different from those obtained in C_6D_6. The use of $Pr(facam)_3$ in both benzene (d_6) and $CDCl_3$ resolved the N-CH_3 resonance peaks of the d and l isomers in addition to the C-CH_3 and the CH-O resonance peaks. The separation appears to be much better in benzene (d_6) than in $CDCl_3$. In addition the differential shift of the l-N-CH_3 and d-N-CH_3 resonance peaks is greater in C_6D_6 than in $CDCl_3$. Unfortunately, benzene (d_6) is not a good solvent for pseudo ephedrines with regard to solubility and cannot be used for their routine analysis. A mixture of $CDCl_3$ and C_6D_6 (1:1) is the best solvent for this purpose.

FIG.2:9 Δ LIS FOR THE N-CH_3 & C-CH_3 SIGNALS OF dl-ψ-EPHEDRINE IN THE PRESENCE OF Pr(facam)$_3$ IN C_6D_6

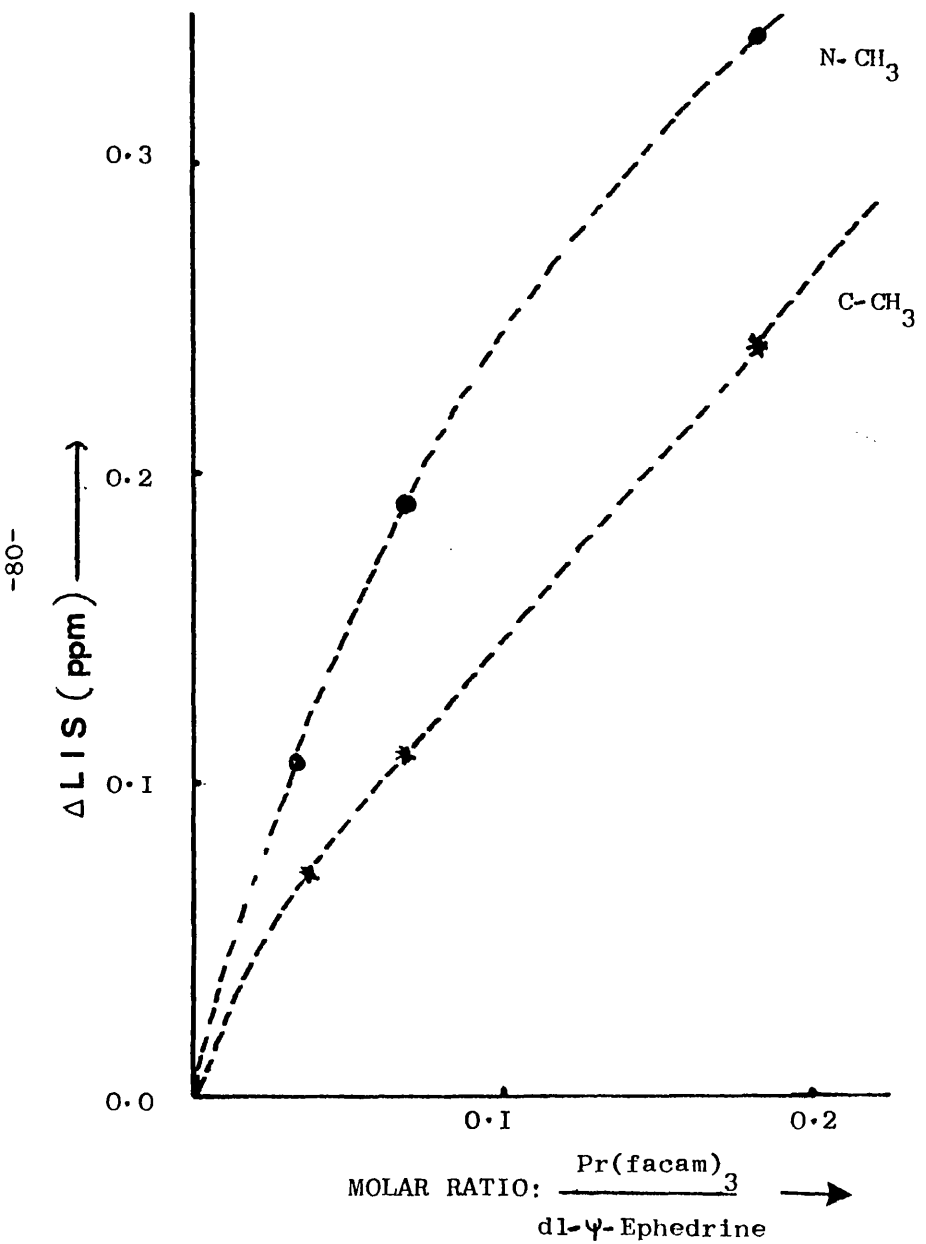


FIG.2:10 EFFECT OF DILUTION ON THE LIS OF PSEUDO EPHEDRINE AT A FIXED MOLAR RATIO OF 0·275

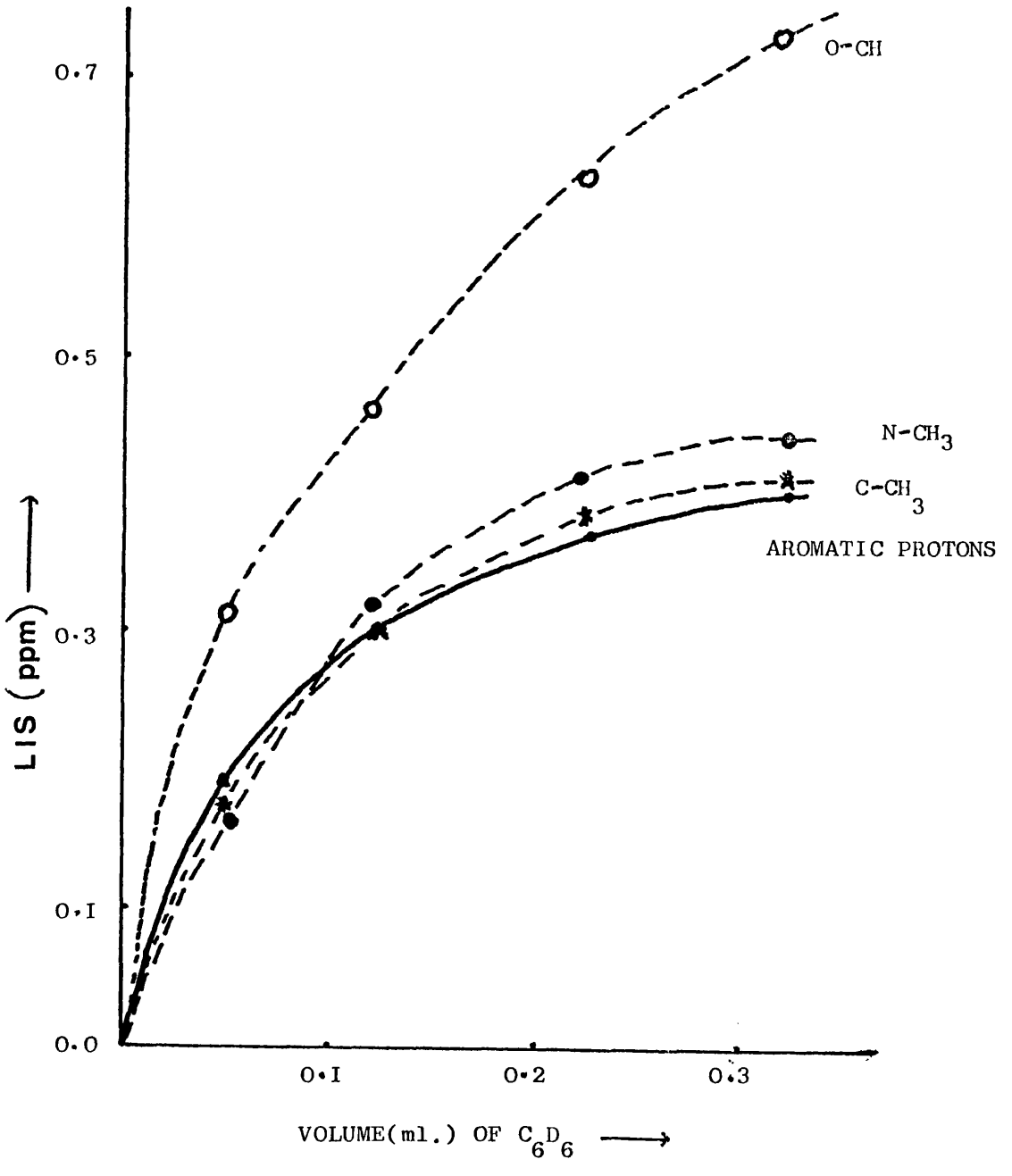
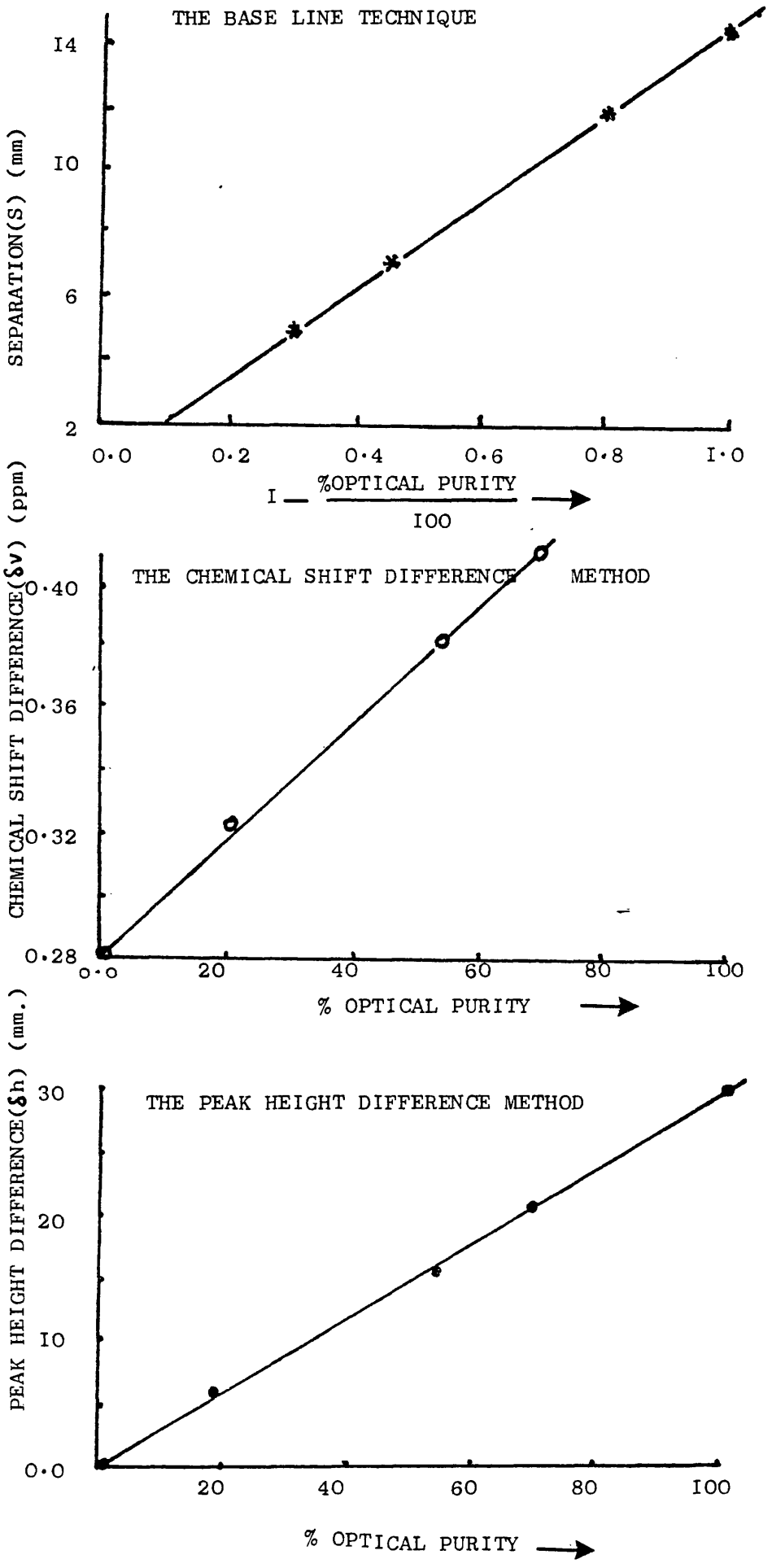


FIG. 2:II OPTICAL PURITY DETERMINATION OF PSEUDO EPHEDRINE



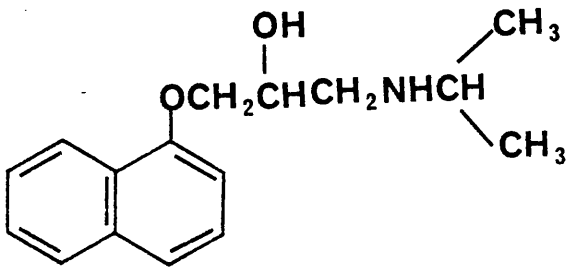
The effect of dilution on the LIS of the various proton groups in pseudo ephedrine was similar to that of ephedrine. The results are shown in Figure 2:10 (page 80) and in table 2:10 (page 78).

Optical Purity determination:

Like ephedrine, the pseudo ephedrine optical purity determination by the base line technique, chemical shift difference and the peak height difference methods gave linear graphs. The results are given in table 2:9A (page 76) and in figure 2:11 (page 81).

Using $Pr(facam)_3$ in $C_6D_6/CDCl_3$ (1:1) well resolved signals were obtained for the N-CH_3 at a molar ratio of 0.075. It was therefore convenient to use peak height measurement at that molar ratio for the optical purity determination. The experiment was successful up to optical purity of about 30%. There was a constant error of about 13% in all levels of optical purity up to 28.1%. The results are given in table 2:9B. It is clear that the new techniques are superior to peak height measurement.

2.2.3 PROPRANOLOL



(2:6)

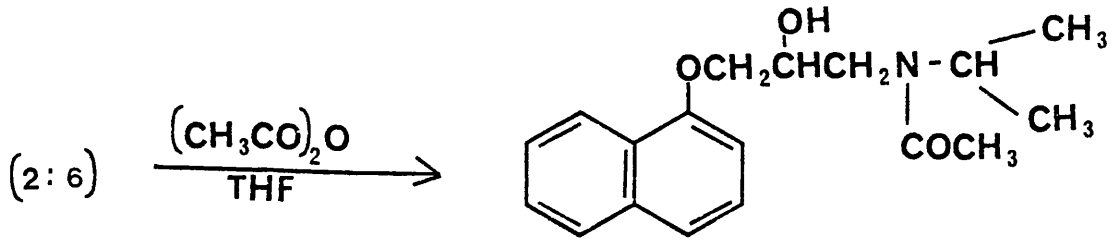
Propanolol (1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol) (2:6) is a β-adrenergic receptor blocking agent developed by Black *et al.*[118]. It is extensively used in the treatment of hypertension.

Propanolol is a mixture of (+) and (-) isomers[119] with only the (-) isomer having β-blocking activity[120]. The enantiomeric purity of propranolol has been determined by a combination of gas chromatography and mass spectrometry using a deuterium labelling technique[120].

In this work, the optical purity of propranolol has been determined using the *N*-acetyl derivative (2:7) and a chiral lanthanide shift reagent ($Eu(facam)_3$). The optical purity determination was preceeded by the determination of the ΔLIS of the $COCH_3$ belonging to (2:7). The results are given in tables 2:11 A and B (page 84) and in figures 2.13A and B (page 89).

2.2.3.1 Results and Discussion

The addition of shift reagents to propranolol resulted in broadening of bands. There was no visible differential shift for the d and l isomers. In view of this, it was necessary to use a derivative for the optical purity determination. The *N*-acetyl derivative (2:7) proved suitable for the purpose. The equation for the synthesis of (2:7) is given in Scheme 2:1. The yield (see section 2.6) was



(2:7)

Scheme 2:1

Table 2:11A $COCH_3$ of *N*-acetylpropranolol ΔLIS in CCl_4 using $Eu(facam)_3$

[*N*-acetylpropranol] = 0.25M

*MOLAR RATIO	(+) isomer		(-) isomer		ΔΔv
	δ	Δv	δ	Δv	(ppm)
0.00	2.10	0.00	2.10	0.00	0.00
0.183	3.07	0.97	2.92	0.82	0.15
0.276	3.64	1.54	3.42	1.32	0.22
0.367	4.15	2.05	3.87	1.77	0.28
0.521	4.70	2.60	4.39	2.29	0.31

Table 2:11B $COCH_3$ of *N*-acetyl propranolol ΔLIS in $CDCl_3$ and C_6D_6 using $Eu(facam)_3$

n-acetylpropanolol = 0.25 M

C_6D_6		$CDCl_3$	
*MOLAR RATIO	ΔΔv (ppm)	MOLAR RATIO	ΔΔv (ppm)
0.00	0.00	0.00	0.00
0.159	0.10	0.189	0.00
0.260	0.12	0.404	0.00
0.357	0.17	0.465	0.01
0.493	0.15	0.540	0.02

$$\text{* MOLAR RATIO} = \frac{Eu(facam)_3}{\text{dl-}N\text{-acetylpropranolol}}$$

Table 2:12 Optical purity determination of propranolol using the *N*-acetylderivative

[*N*-acetylpropranolol] = 0.248M

Reagent: $Eu(facam)_3$

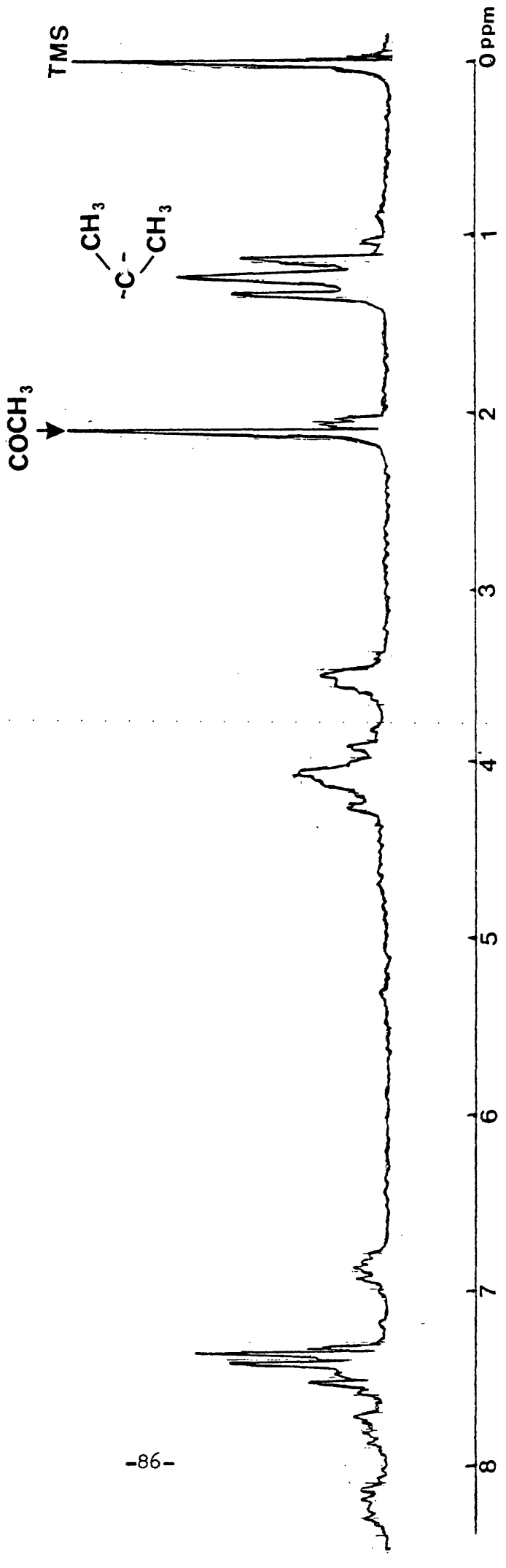
*Molar Ratio: 0.40 ; solvent: CCl_4

Analytical Peak: OCH_3 signals at δ3.9 - 4.2 ppm

% Optical Purity	$1 - \frac{\%OP}{100}$	Separation (S) (mm)	Chemical Shift difference (ppm)	Peak height difference (d-l) (mm)
0.0	1.00	4.9	0.24	-0.5
24.9	0.75	3.9	0.26	3.3
50.4	0.50	2.8	0.29	8.6
75.4	0.25	1.4	0.31	15.9
100.0	0.00	0.2	-	21.4

$$\text{MOLAR RATIO} = \frac{Eu(facam)_3}{N\text{-acetylpropranolol}}$$

FIG. 2:12 N-Acetylpropranolol (0·25M) in CCl_4

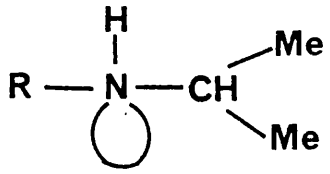


low. This might be due to the formation of the ester in addition to the amide since the OH was not protected from acetylation.

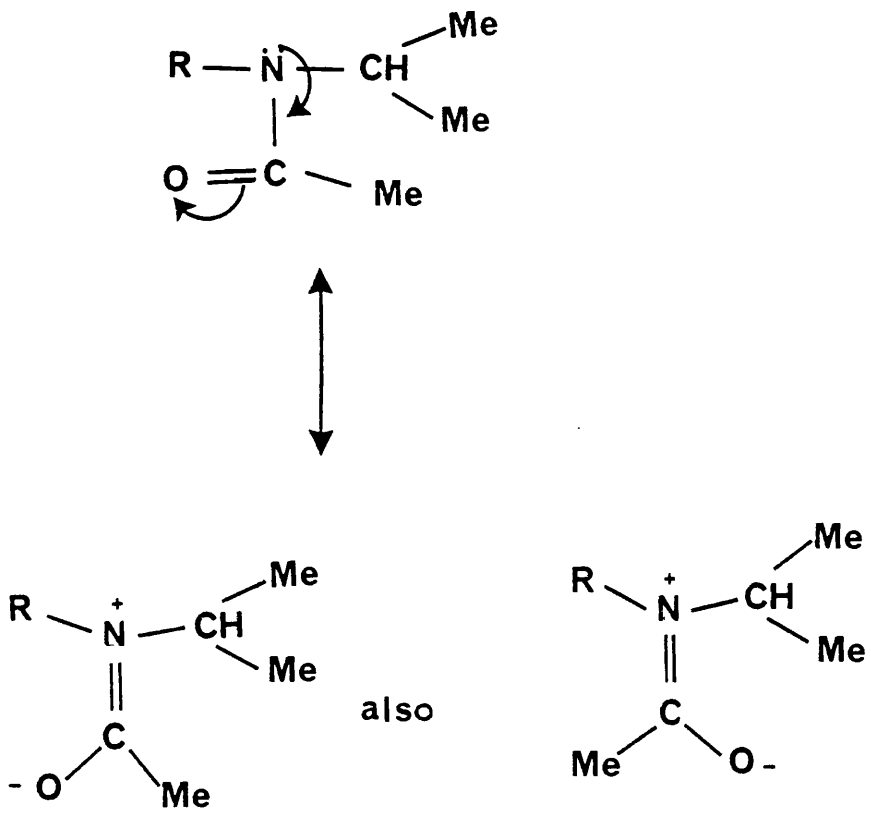
Non-equivalence of the isopropyl group:

The nmr spectrum of *N*-acetylpropranolol (figure 2.12, page 86) reveals non-equivalence of the isopropyl methyl groups ($CH\langle^{CH_3}_{CH_3}$). In the spectrum of propranolol itself, a doublet was obtained for the two methyls indicating equivalence of the two groups.

The equivalence of the isopropyl groups in propranolol is probably due to the fact that the lone pair electrons on the nitrogen are localized, and moreover, there is free rotation at the N-CH bond. On the other hand, when



acetylated the electrons are delocalized as shown below. The populations of the various conformers are not equal.



The non-equivalence of the isopropyl group in certain molecules has already been observed by many workers[121-127]. Four possible explanations have been proposed[121-127].

(1) Different conformers;

(2) Anisotropic effect of the carbonyl (C=O) group;

(3) Steric hindrance to rotation at the N - C bond;

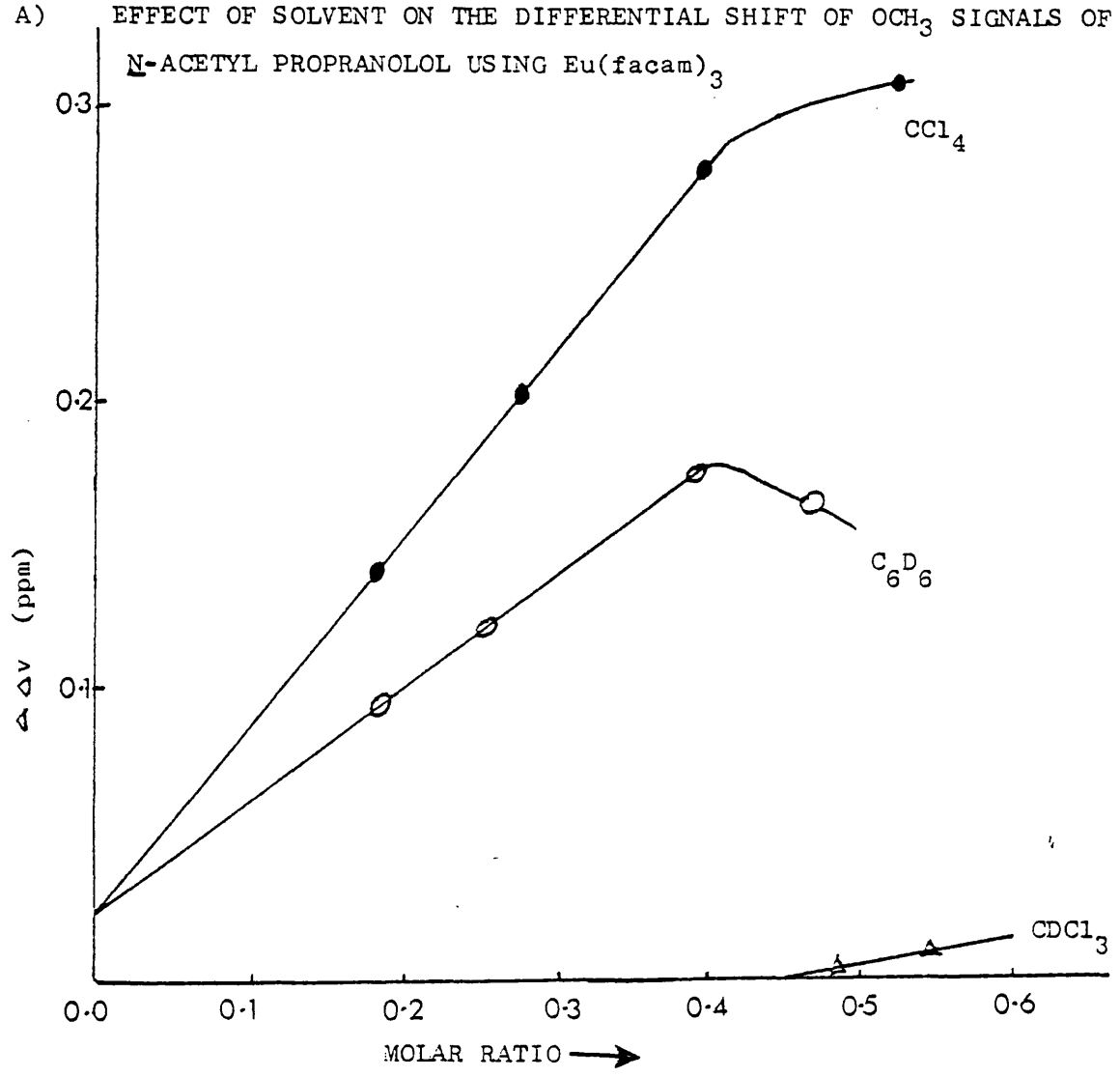
(4) Inter/Intra molecular hydrogen bonding.

The isopropyl group methyl resonance could not be used for the purpose of quantification in view of the non-equivalence.

ΔLIS of the $COCH_3$ signal:

Carbon tetrachloride proved to be the best solvent among the solvents employed (see figure 2.13A, page 89). A differential

FIG.2:13 A) EFFECT OF SOLVENT ON THE DIFFERENTIAL SHIFT OF OCH_3 SIGNALS OF N-ACETYL PROPRANOLOL USING $Eu(facam)_3$



B) OCH_3 LIS OF THE (+) & (−) N-ACETYL PROPRANOLOL IN CCl_4 USING $Eu(facam)_3$

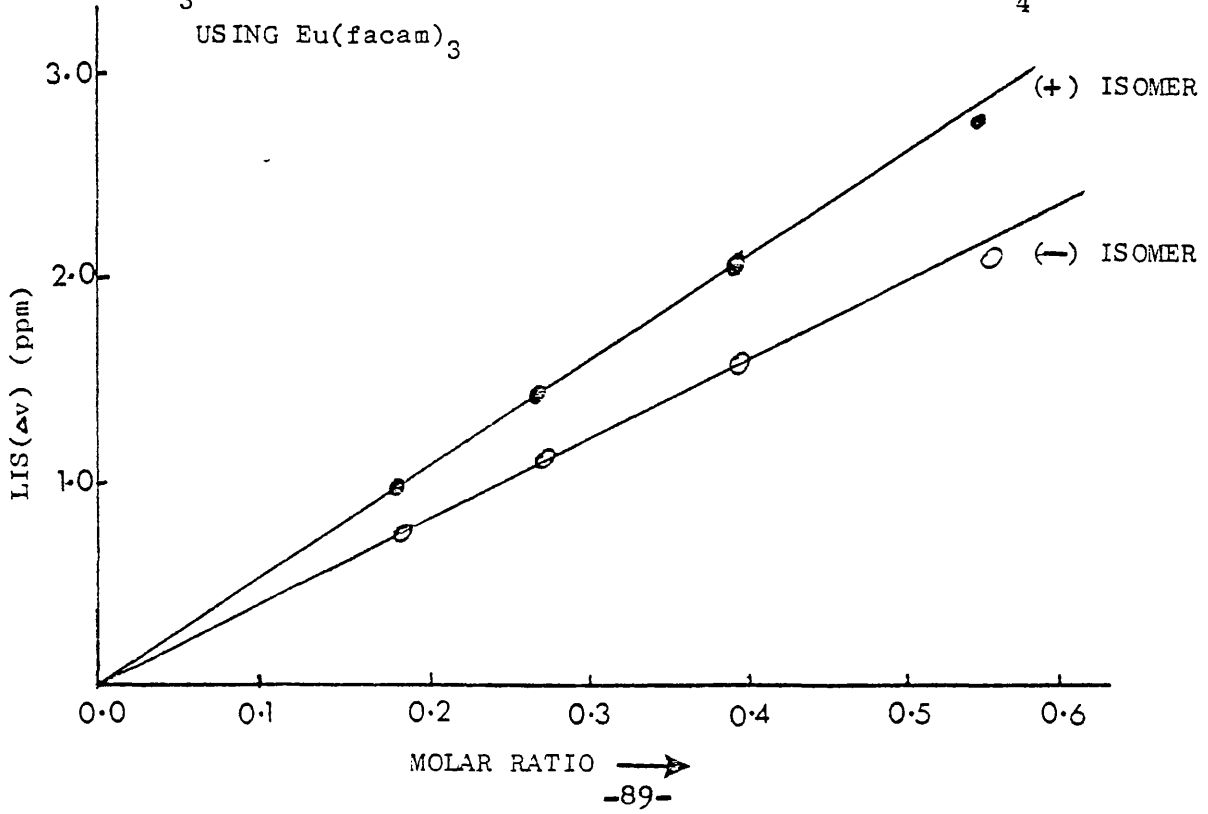
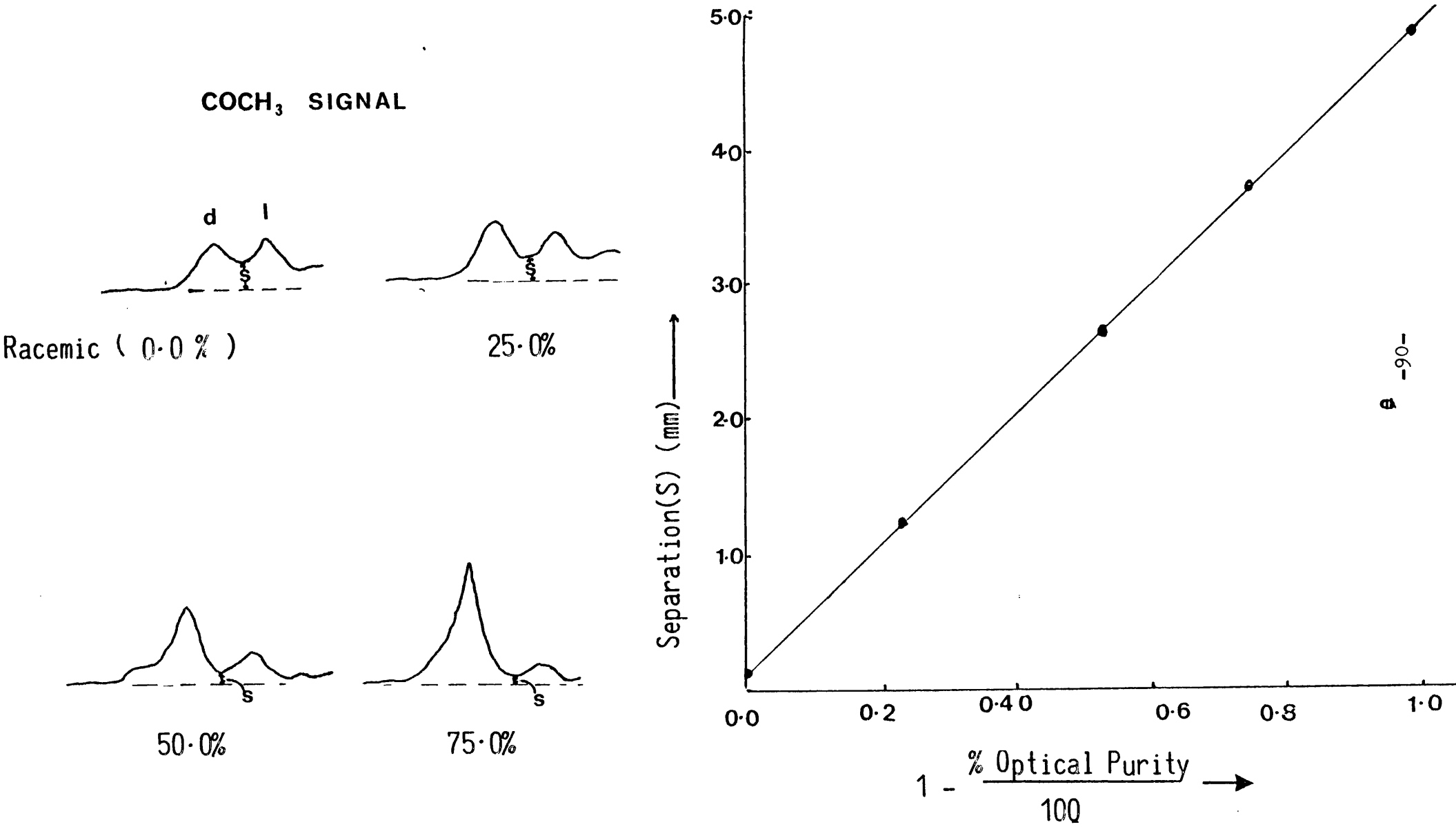


FIG. 2:14 The Base Line Technique for Optical purity determination of Propranolol



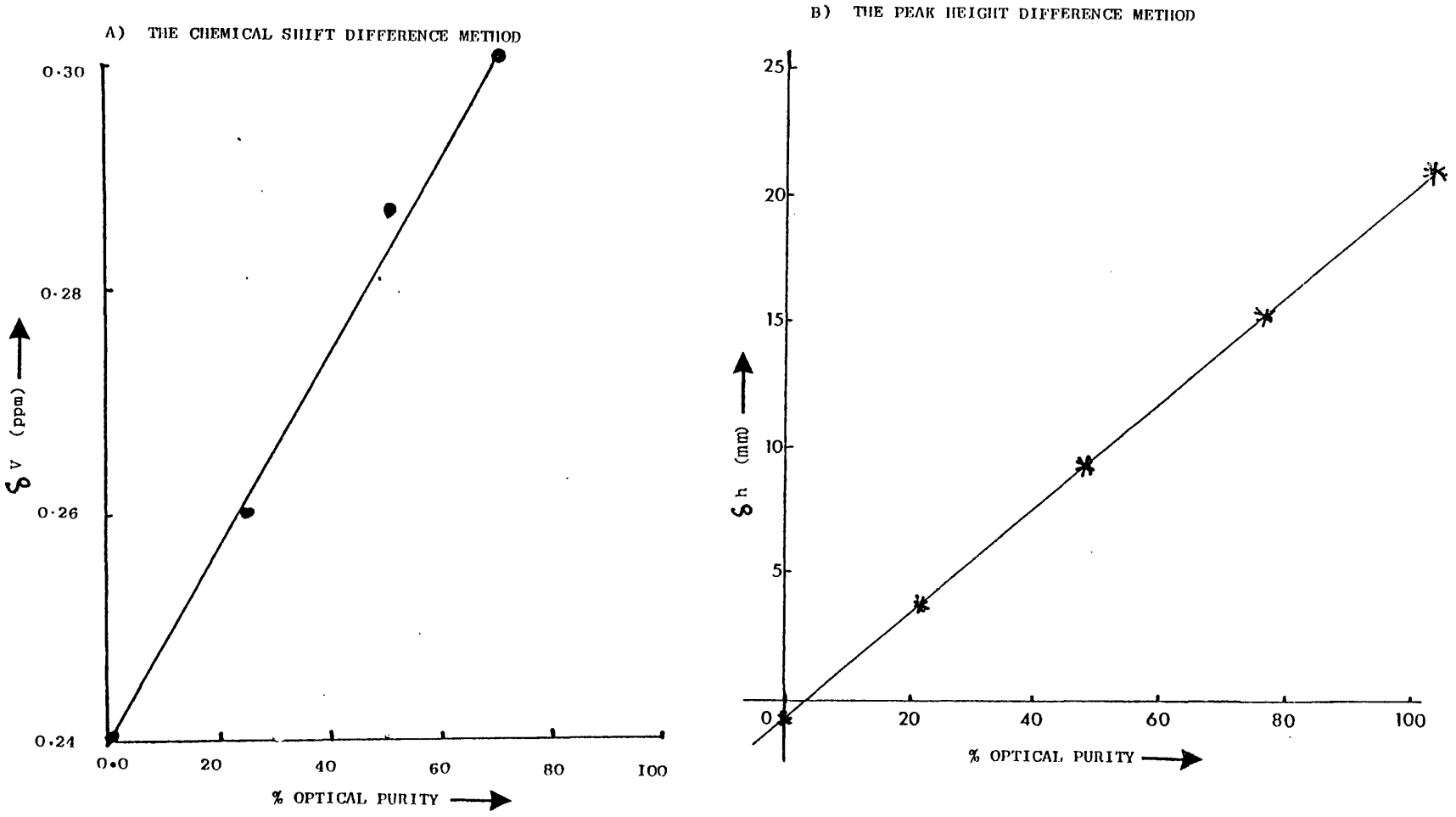


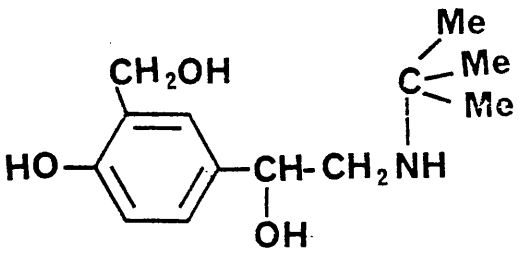
FIG. 2:15 THE OPTICAL PURITY DETERMINATION OF PROPRANOLOL

shift of over 0.3 ppm was obtained in CCl_4 at low molar ratio. The (+) isomer gave a larger LIS than the (-) isomer (figure 2.13B, page 89). As seen in figure 2.13A, $CDCl_3$ was not a suitable solvent. From the LIS plots (figures 2.13A and B), it appears that the Eu(facam)$_3$ and the *N* -acetylpropranolol forms a 1:2 chelate.

Optical purity determination:

Figures 2:14 and 2:15, and table 2:12 show the results of the optical purity determination by the base line technique, the chemical shift difference method and the peak height difference method. A linear graph was obtained in each case, indicating the accuracy of the methods. Though a large $\Delta\Delta\nu$ was obtained, complete separation of the peaks was not possible in view of the broadness of the peaks at higher molar ratios. For the same reason, integration and direct peak height measurements were not suitable. The simplicity of the base line technique, the chemical shift difference or peak height difference methods coupled with their accuracy makes them well suited for the optical purity determination of propranolol.

2.2.4 SALBUTAMOL



(2:8)

Salbutamol (2:8) is a sympathomimetic (selective β_2) amine used as a bronchodilator. It is extensively employed in the treatment of asthma.

The optical purity of salbutamol has been determined through the formation of its silyl derivative (2:8) and the subsequent application of the chiral lanthanide shift reagent Eu(facam)$_3$ and the base line technique, the chemical shift difference and the peak height difference methods. CCl_4 was the solvent employed in the analysis.

Silylation has a wide range of applications in analytical chemistry. Two of its applications - blocking of reactive sites and altering of the solubility of materials[128-131], have been employed here to aid the optical purity determination of salbutamol.

The direct use of CLSR for the optical purity determination of salbutamol proved impossible because of the presence of the amino group and the three hydroxy groups in the molecule (see section 2.1). Salbutamol is not readily soluble in the common organic solvents used for optical purity determinations when CLSRs are used. Salbutamol is freely soluble in water and this makes its extraction from aqueous solution or plasma[132] difficult. For these reasons, the analysis of salbutamol has been performed after conversion to a TMS derivative (2:10).

The equation for the silylation is shown in Scheme 2:2. The SiOCH_2 signal was employed as the analytical peak. The results

Table 2:13 Determination of the ΔLIS of CH_2OSi signal of (2:1O) in different solvents.

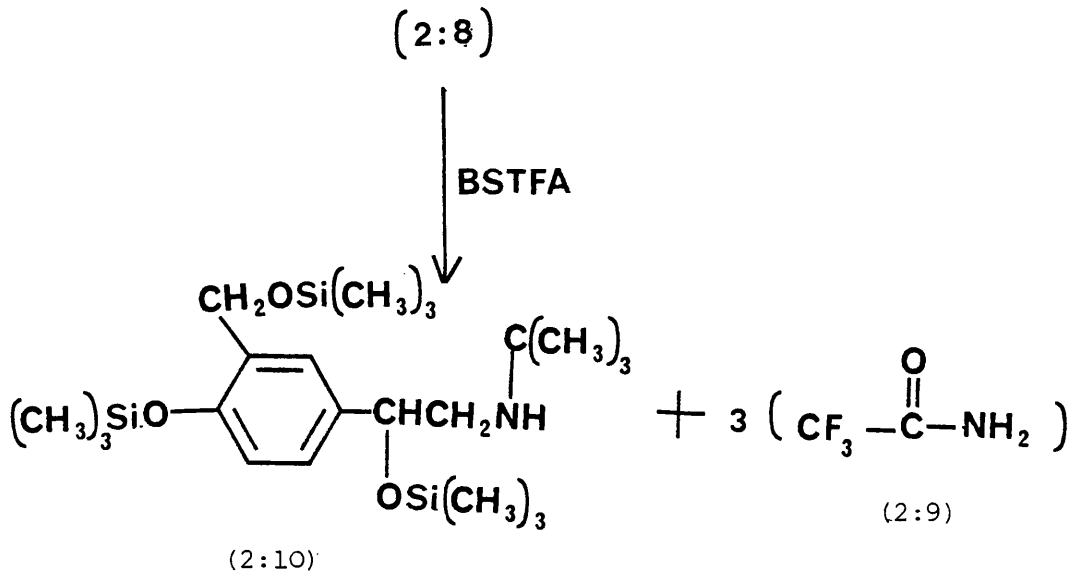
MOLAR RATIO $\frac{Eu(facam)_3}{Salbutamol}$	CH_2OSi ΔΔv in Hz $CDCl_3$	CCl_4	C_6D_6
0.4	1.6	2.3	3.3
0.6	2.4	3.5	4.0
0.8	3.2	4.7	4.8
1.0	4.0	-	5.7
1.2	4.8	7.8	6.5
1.4	5.6	8.1	7.6
1.6	-	-	8.2

Table 2:14 The optical purity determination of Salbutamol

Shift reagent: $Eu(facam)_3$
Solvent: CCl_4 (0.7 ml)
*Molar ratio: 1.20
Analytical peak CH_2OSi signals at δ4.6 - 5.4 ppm

% Optical purity(OP)	$1 - \frac{\%\ OP}{100}$	Separation (S; mm)	Chemical shift difference (δv; ppm)	Peak height difference (R - S; mm)
0.0	1.0	7.0	0.10	0.9
25.0	0.75	5.2	0.14	8.0
50.0	0.50	3.7	0.17	15.6
75.3	0.25	1.8	0.19	22.2
100.0	0.00	0.15	-	30.2

*Molar ratio was based on the weight of salbutamol (60mg), [Reagent] / [Salbutamol]



Scheme 2:2

for the ΔLIS and the optical purity determinations are shown in tables 2:13 and 2:14 (page 94) and in figures 2:17A and B, 2:18 and 2:19 (pages 99-100).

2.2.4.1 Results and Discussion

The reaction of BSTFA with salbutamol gives two products - $(O\text{-TMS})_3$ salbutamol (2:10) and Trifluoroacetamide (2:9). It is necessary that all the trifluoroacetamide be removed from the product by distillation before the use of the chiral shift reagent since trifluoroacetamide competes with the substrate for the shift reagent, thus decreasing the sensitivity of the method. For the same reason, all the excess BSTFA must be removed (see section 1.2).

It is interesting to note that whereas in $CDCl_3$ and in CCl_4 the 3 O-TMS groups have different chemical shifts and therefore appear as three singlets; in C_6D_6 all the 3 O-TMS groups are

equivalent and appear as a singlet. The nmr spectrum of (2:10) in CCl_4 is shown in figure 2:16 (page 97).

Lanthanide induced shifts (LIS):

Two singlets were obtained for the CH_2OSi signal when the Eu(facam)$_3$ was added, indicating the resolution of the S(+) and R(-) isomers. There was, however, no appreciable LIS for the CH_2OSi signal even after a large quantity of the shift reagent had been added.

Large LIS's were obtained with the $C(CH_3)_3$, CH, CH_2N and NH groups. No observale LIS's were obtained with the $(CH_3)_3Si$ groups. These observations show that the co-ordination centre is at the nitrogen atom where steric hindrance is minimum. There was no indication of any binding at the oxygen atoms of the O-TMS groups. The signals of those protons distant from the binding site remained sharp throughout the experiment, whereas those nearer to the nitrogen atom gave broad and featureless resonances.

The S(+) salbutamol gave larger LIS than the R(-) isomer (figure 2:17B, page 98).

Optical purity determination:

Of the three solvents tested ($CDCl_3$, C_6D_6 and CCl_4), CCl_4 proved to be the most suitable, though benzene (d_6) gave sharper peaks. Figure 2:17A (page 98) shows the solvent effect on the differential shifts. Two chiral shift reagents - Pr(facam)$_3$ and Eu(facam)$_3$ - were used for the preliminary analysis but only the

FIG. 2:16 $(O\text{-}TMS)_3$-SALBUTAMOL IN CCl_4

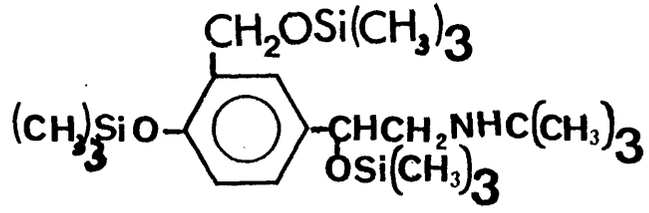
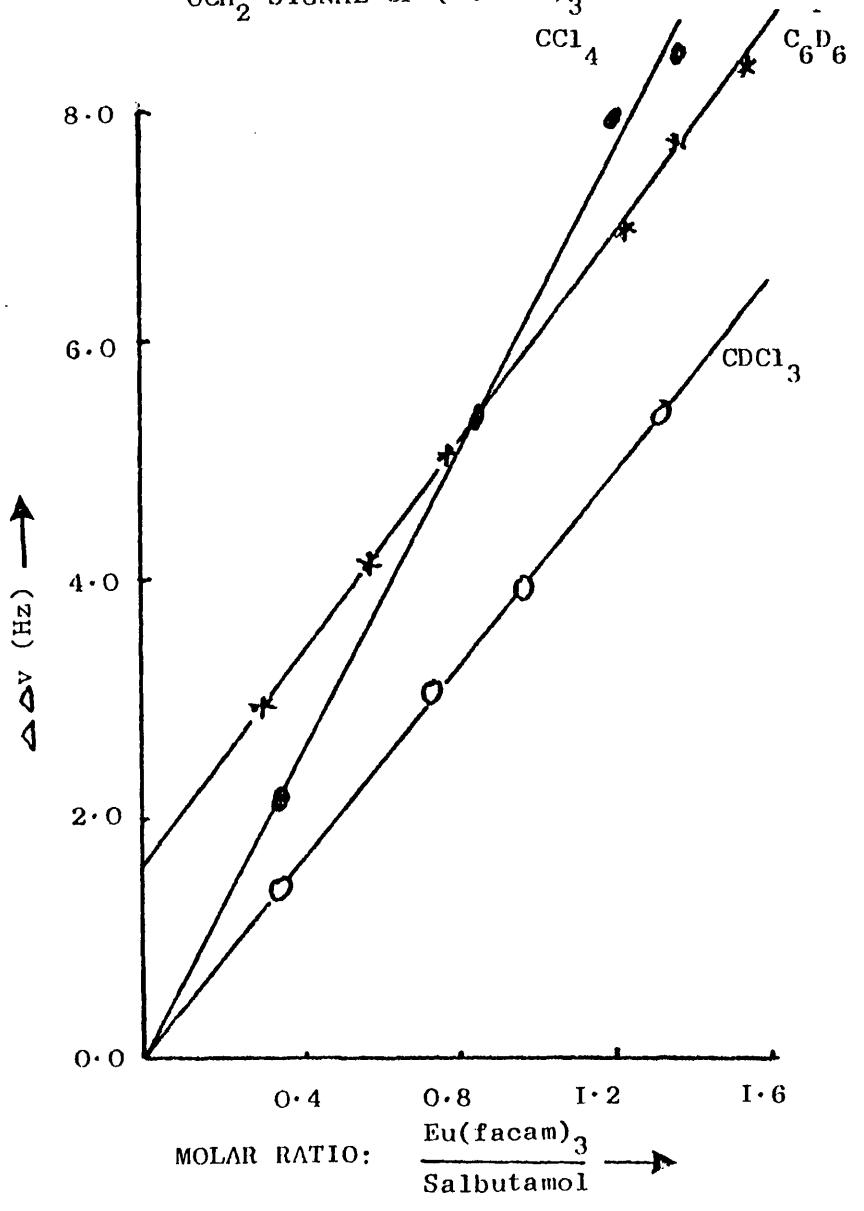


FIG. 2.17

A) SOLVENT EFFECT ON THE DIFFERENTIAL SHIFT OF THE OCH_2 SIGNAL OF (O TMS)$_3$ SALBUTAMOL



B) LIS OF THE OCH_2 SIGNAL FOR THE R(-) & S(+) (O TMS)$_3$ SALBUTAMOL IN CCL_4

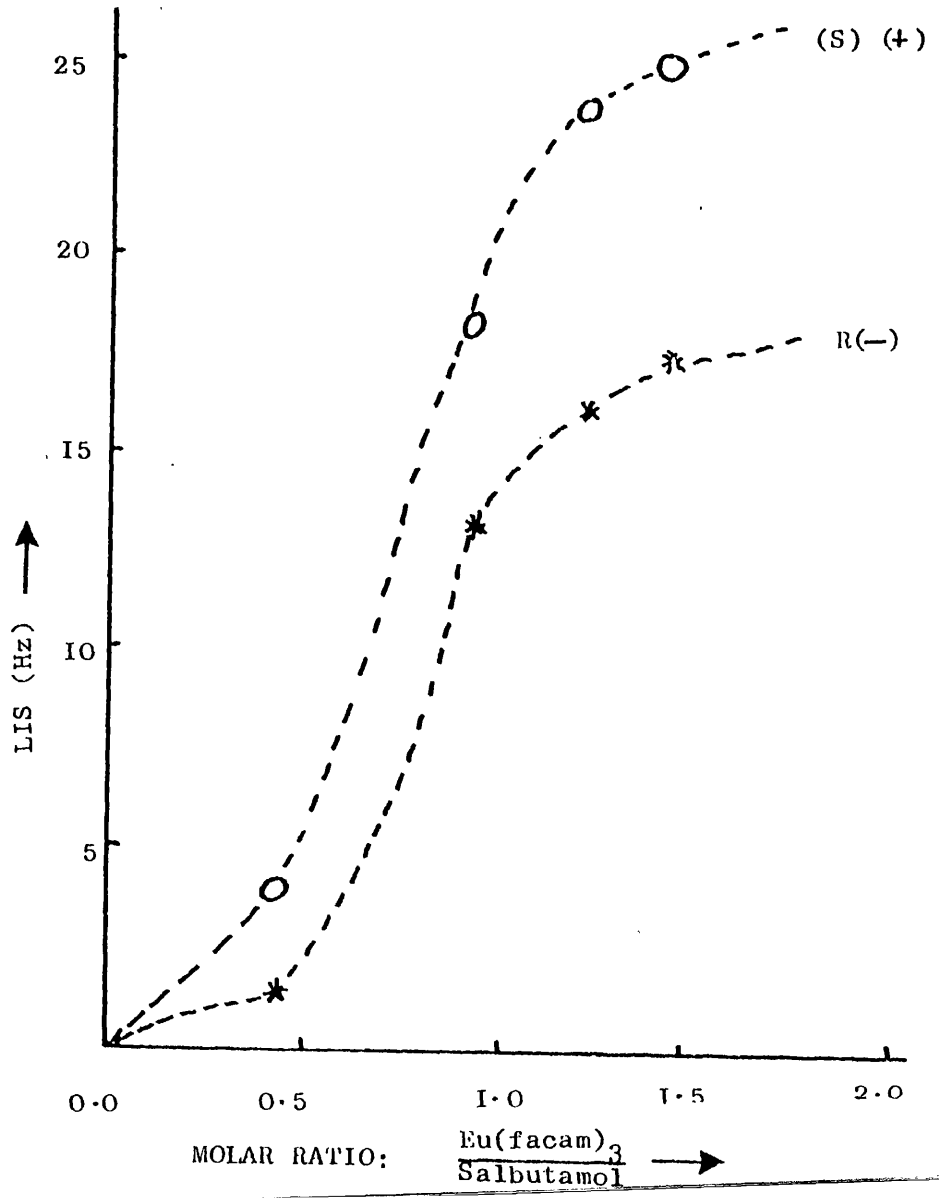


FIG. 2:18 The Base line technique for Optical purity determition of Salbutamol

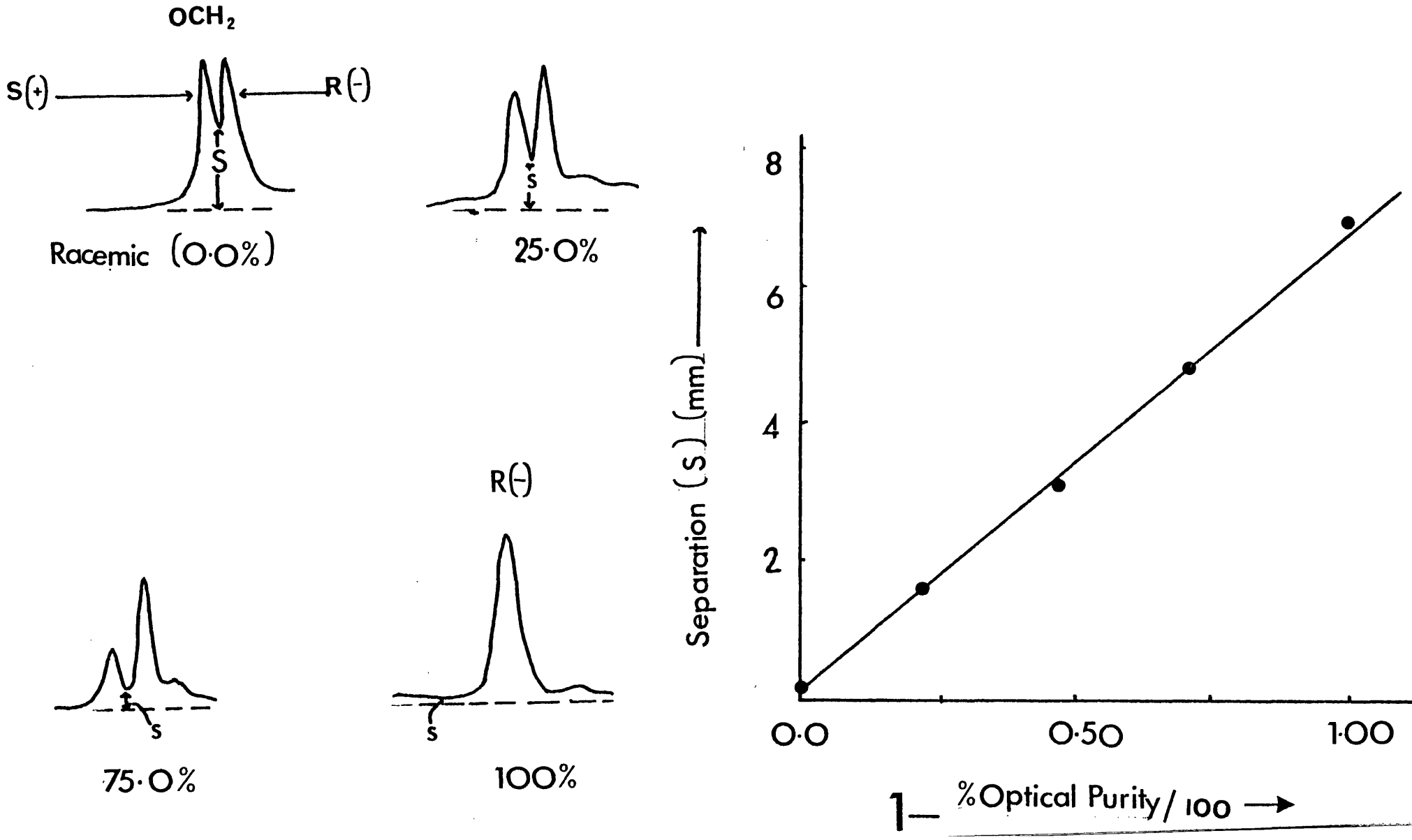
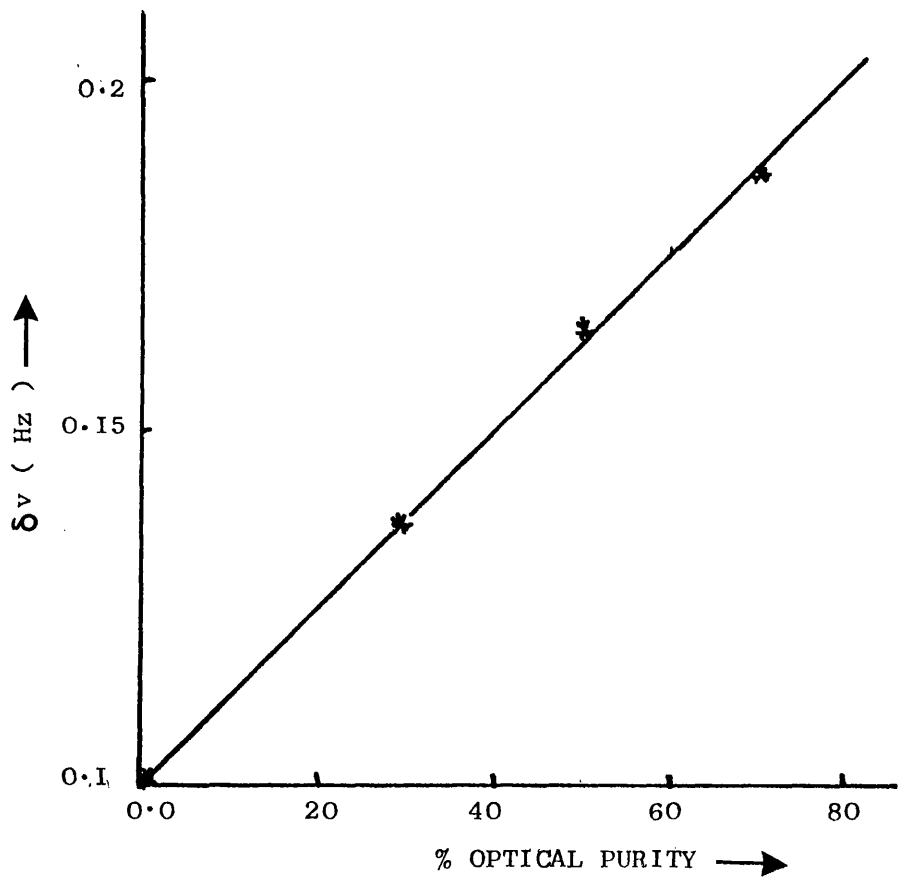
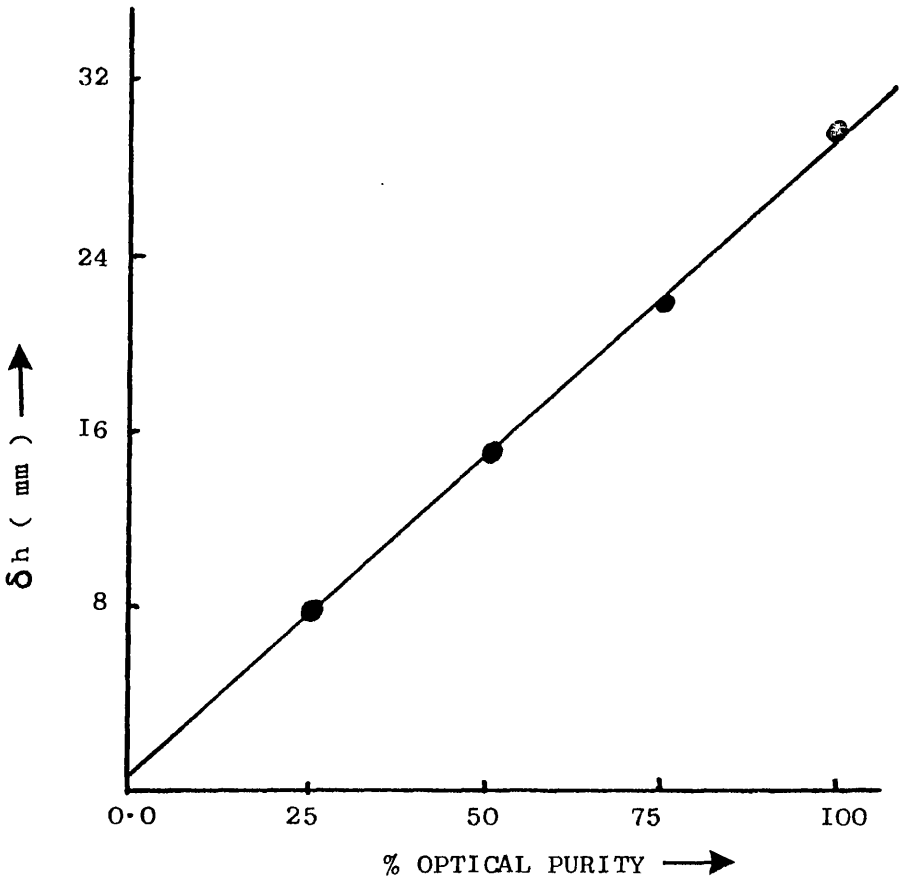


FIG. 2:I9 OPTICAL PURITY DETERMINATION OF SALBUTAMOL

A) CHEMICAL SHIFT DIFFERENCE METHOD

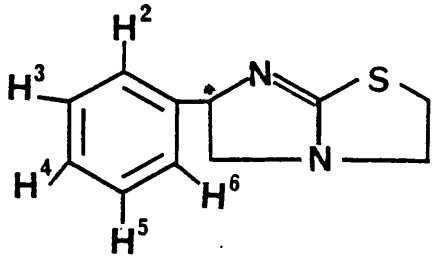


B) PEAK HEIGHT DIFFERENCE METHOD



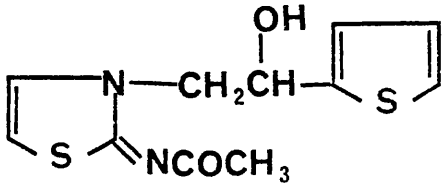
$Eu(facam)_3$ gave separation of the CH_2OSi resonances. As with the use of almost all chiral shift reagents, slight broadness of the peaks at higher molar ratios was observed. Complete separation was not achieved. The base line technique, the chemical shift difference method and the peak height difference method were used for the analysis. Linear plots were obtained for all the methods indicating the suitability of the methods for the optical purity of salbutamol. The results are given in table 2:14 (page 94) and in figures 2:18 and 2:19 (pages 99 & 100).

2.2.5 TETRAMISOLE



(2:11)

Tetramisole (6-phenyl-2,3,5,6-tetrahydroimidazo(2,1-*b*)thiazole) (2:11) is a broad-spectrum antihelmintic, active against nematodes[133-136]. It was developed as a direct result of metabolic investigations on another compound, 2-acetylimino-3-[2-hydroxy-2-(2-thienyl)ethyl] thiazoline (Thiazothenol (2:12)) which was studied as an antihelmintic in sheep and poultry.



(2:12)

As synthesized, tetramisole is a racemic mixture. The anthelmintic activity is almost exclusively the property of the S(-) isomer - the S(-) isomer is twice as potent as the racemate and several times as active as the R(+) form. The toxicity of the two isomers is equal. Thus the relative safety of the S(-) isomer is about twice that of the racemate[133,134].

Chemists at Janssen Laboratories established the absolute configuration of the isomers by synthesis from the optically active intermediate phenylethylenediamines[134], and the American Cyanamid Company group resolved the racemic mixture[135].

Tetramisole hydrochloride is readily soluble in water and is said to be effective by oral, subcutaneous, intramuscular and intraperitoneal administration.

Since the anthelmintic activity of tetramisole depends exclusively on the S(-) isomer (levamisole), it is important that the optical purity be determined. In this work, a fast and simple method has been developed by the use of the chiral shift reagent $Eu(facam)_3$ in $CCl_4/CDCl_3$ (5:2) using nmr. This involves integration of the *C*H* peaks for the (+) and (-) isomers (i.e. dexamisole and levamisole respectively). The peak height difference (δh) method was also employed for the analysis. The results are shown in table 2:16 (page 105) and a plot of the peak height difference (δh) *versus* the % optical purity is shown in figure 2:23 (page 111). The LIS's were also determined using $Eu(fod)_3$, $Pr(fod)_3$ and $Eu(facam)_3$. The results for this determination are given in tables 2:15A - C (pages 103 and 104).

Table 2:15A. LIS of tetramisole using $Eu(fod)_3$ in $CCl_4/CDCl_3$ (5:2)

[Tetramisole] = 0.24 M

MOLAR RATIO $Eu(fod)_3$ / Tetramisole	*CH δ	*CH Δv	Aromatic protons H_2 & H_6 δ	Aromatic protons H_2 & H_6 Δv	Aromatic protons H_3,H_4 & H_5 δ	Aromatic protons H_3,H_4 & H_5 Δv
0.0	5.35	0.0	7.22	0.0	7.22	0.0
0.062	6.08	0.73	7.75	0.53	7.25	0.03
0.099	6.54	1.19	8.00	0.78	7.30	0.08
0.183	7.53	2.18	8.85	1.63	7.38	0.16
0.342	9.78	4.43	10.05	2.83	7.54	0.32

Table 2:15B. LIS of tetramisole using $Pr(fod)_3$ in $CCl_4/CDCl_3$ (5:2)

[Tetramisole] = 0.26 M

MOLAR RATIO $Pr(fod)_3$ / Tetramisole	*CH δ	*CH Δv	Aromatic protons H_2 & H_6 δ	Aromatic protons H_2 & H_6 Δv	Aromatic protons H_3,H_4 & H_5 δ	Aromatic protons H_3,H_4 & H_5 Δv
0.00	5.35	0	7.22	0.0	7.22	0.0
0.047	4.09	-1.26	6.37	-0.85	6.99	-0.23
0.106	+		5.05	-2.17	6.72	-0.50
0.189	+		3.35	-3.85	6.32	-0.90

+ *CH resonance peak merged with other resonances.

Table 2:15C LIS and ΔLIS of tetramisole using $Eu(facam)_3$

[Tetramisole] = 0.368 M

Solvent: $CDCl_3/CCl_4$ (2:5)

MOLAR RATIO $\frac{Eu(facam)_3}{Tetramisole}$	Aromatic protons H_3, H_4 & H_5		Aromatic protons H_2 & H_6		*CH Dexamisole		*CH Levamisole		ΔΔv (ppm)
	δ	Δv	δ	Δv	δ	Δv	δ	Δv	
0.0	7.22	0.0	7.22	0.0	5.35	0.0	5.35	0.00	0.0
0.052	7.26	0.04	7.67	0.45	6.10	0.75	6.07	0.72	0.03
0.165	7.35	0.13	8.46	1.24	+		+		
0.229	7.45	0.23	8.97	1.75	8.37	3.02	8.22	2.87	0.15
0.390	+		10.03	2.81	10.41	5.06	+		
0.481	+		10.70	3.48	11.75	6.40	11.34	5.99	0.41
0.580	+		11.14	3.92	12.80	7.45	12.24	6.89	0.56
0.638	+		11.43	4.21	13.44	8.09	12.70	7.44	0.65

+ Resonance peaks merged with other resonances.

Table 2:16 Optical purity determination of tetramisole

Shift reagent: $Eu(facam)_3$; Molar ratio Reagent / Sample = 0.64

Solvent: $CCl_4/CDCl_3$ (5:2); † Sample = 0.368 M

Analytical peaks: *C*H* signals at δ13.4 - 14.2 ppm

Actual % Optical Purity	Integration method		Peak Height difference (D-L) (mm)
	% Optical Purity as found by experiment	% Error	
0.0	0.0	-	-0.8
26.5	26.4	0.38	5.2
52.5	52.3	0.38	10.9
69.5	68.9	0.86	14.8
100.0	-	-	21.8

† Mixture of dexamisole and levamisole.

2.2.5.1 Results and Discussion

Lanthanide induced shift:

With the $Eu(fod)_3$, all the shifts were downfield and with the $Pr(fod)_3$ all shifts were upfield. The $Pr(fod)_3$ induced shifts were larger than those of the $Eu(fod)_3$. $Pr(fod)_3$ gave much broader signals than those of $Eu(fod)_3$. In both cases, the largest shift was given by the *C*H* signal indicating that the co-ordination occurs mainly at the imidazo-N = C. Figure 2:21 (page 109) shows a plot of the LIS against the molar ratio of $Eu(fod)_3$ to tetramisole.

In the nmr spectrum of tetramisole (figure 2:20A, page 108) only two resonance peaks could be assigned to particular groups of hydrogen, the aromatic protons at δ7.25 and the *C*H* signal at δ5.65 ppm. The other protons displayed considerable signal overlap. Addition of few milligrams of a shift reagent revealed the presence of three single protons in the 3 - 6 ppm region, one of which has been identified as the *C*H*. SInce there is no other single proton in the molecule other than the aromatic protons it was thought that the two protons close to the chiral centre might behave as two single non-equivalent protons, as is usually the case[160]. To ascertain this, decoupling experiments were performed using a 100 MHz instrument (Jeol 100). The decoupling technique showed that the triplet due to the *C*H* is in fact two doublets arising from coupling separately with the two protons adjacent to the *C*H* which are non-equivalent. The two non-equivalent methylene protons also couple separately with the *C*H* and with each other giving two doublets each which appear as two triplets due to signal overlap.

Using $Eu(facam)_3$ the l and d resonance peaks for the *C*H* group separated (figure 2:20B, page 108). In view of the large coupling constant (*Ca* 10Hz) and the triplet nature of the resonance, complete separation was obtained at high molar ratio (*Ca* 0.64). At this molar ratio, integration was possible though the resonances were broad.

At a molar ratio of 0.638, the resonance positions of the *C*H* were: dexamisole - δ13.44 and levamisole δ12.70 ppm. The results are given in table 2:15C (page 104) and a plot of the differential shift in figure 2:22 (page 110).

Optical Purity Determination:

The optical purity was determined using a molar ratio $\left[\frac{Eu(facam)_3}{Tetramisole}\right]$ of 0.638. The solvent mixture ($CDCl_3/CCl_4$) was chosen because $CDCl_3$ is a better solvent but CCl_4 gives larger differential shifts. Table 2:15 gives the results obtained for analysis of different mixtures of dexamisole and levamisole using integration and peak height difference methods. The methods are simple, and accurate. The main disadvantage is the low sensitivity of the *CH signal (only one proton). Because of this, optical purities above 70% could not be determined, since a large amount of the sample and $Eu(facam)_3$ was required. In fact, provided enough sample and shift reagent ($Eu(facam)_3$) are available, it is possible to determine optical purities up to 95%.

FIG. 2:20 A) Tetramisole (0·368M) in CCl_4/ $CDCl_3$ (5:2).

B) (A) in the presence of $Eu(facam)_3$; Tetramisole / $Eu(facam)_3$ molar ratio of 0·64.

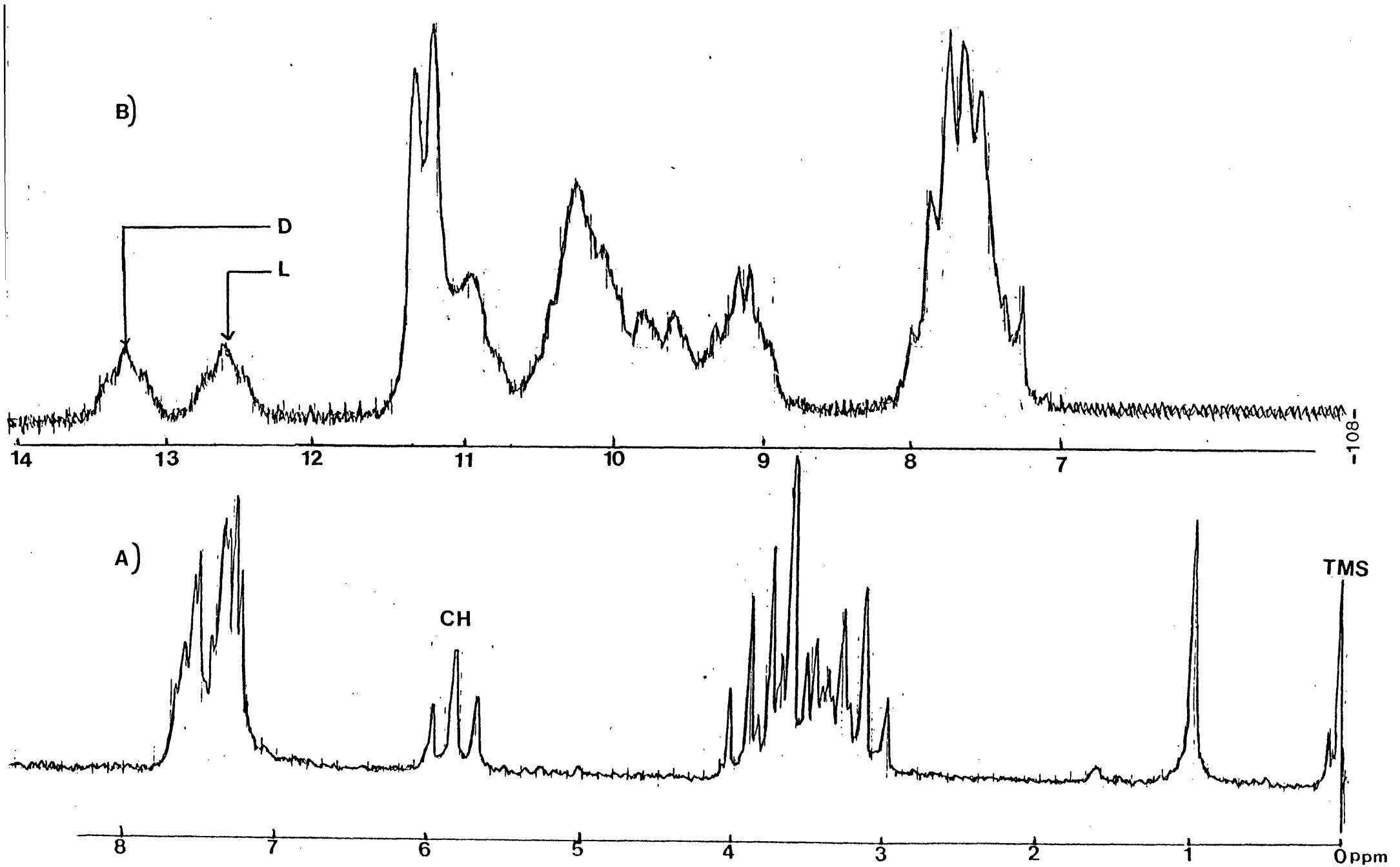
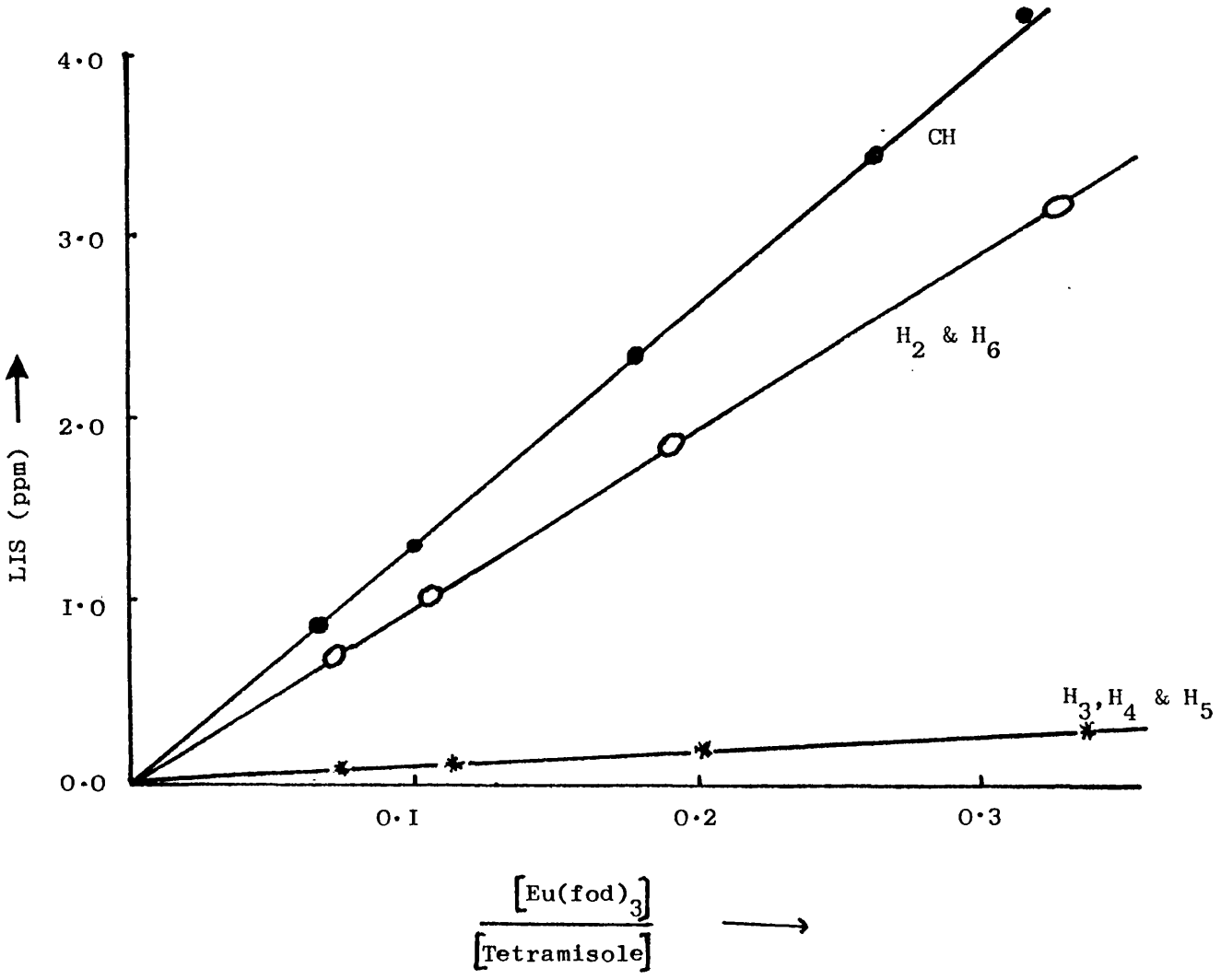

B)
D
L
14
13
12
11
10
9
8
7
108
A)
CH
TMS
8
7
6
5
4
3
2
1
0ppm

FIG. 2:21 TETRAMISOLE LIS VERSUS MOLAR RATIO



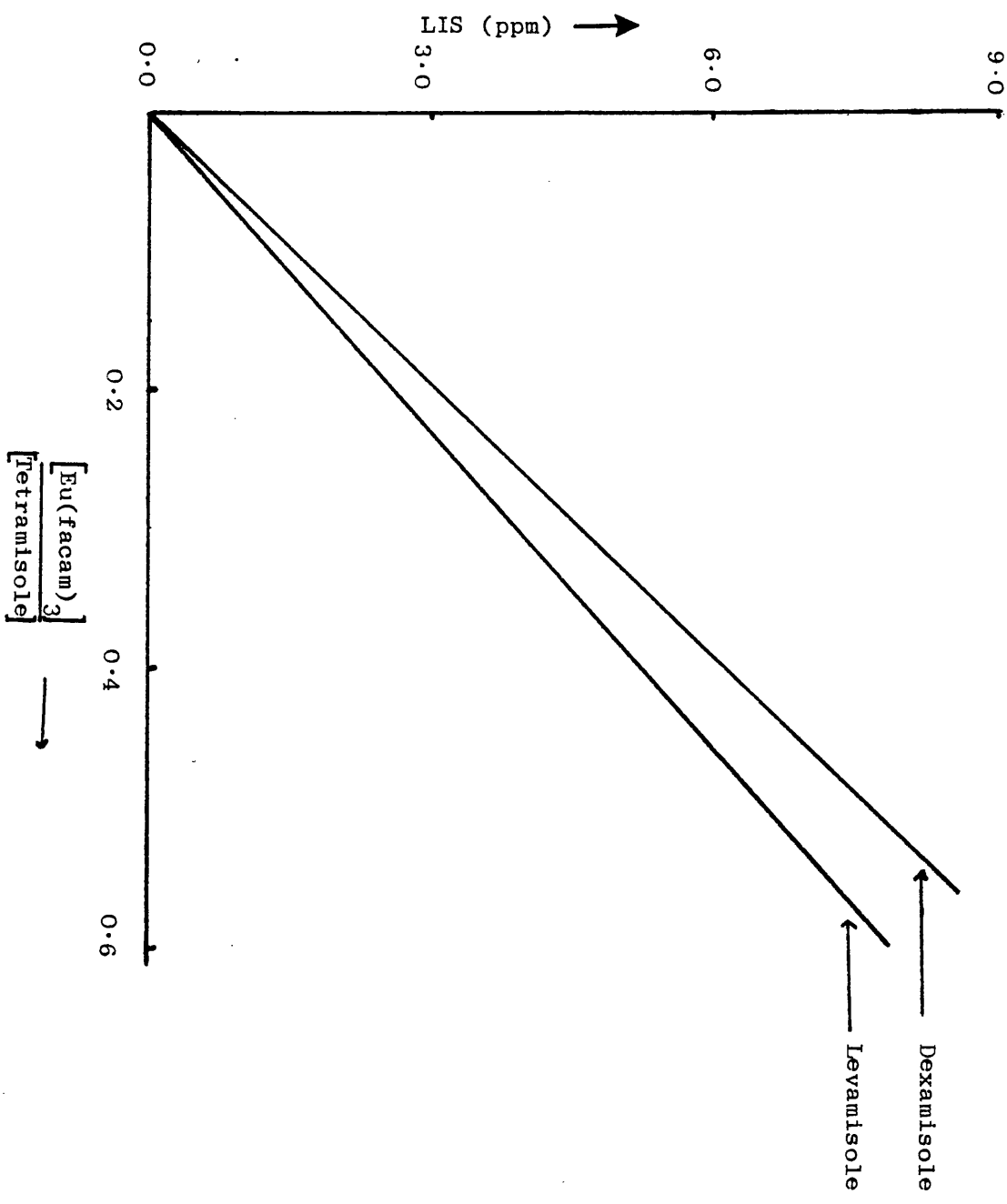


FIG. 2:22 TETRAMISOLE CH LIS VERSUS MOLAR RATIO

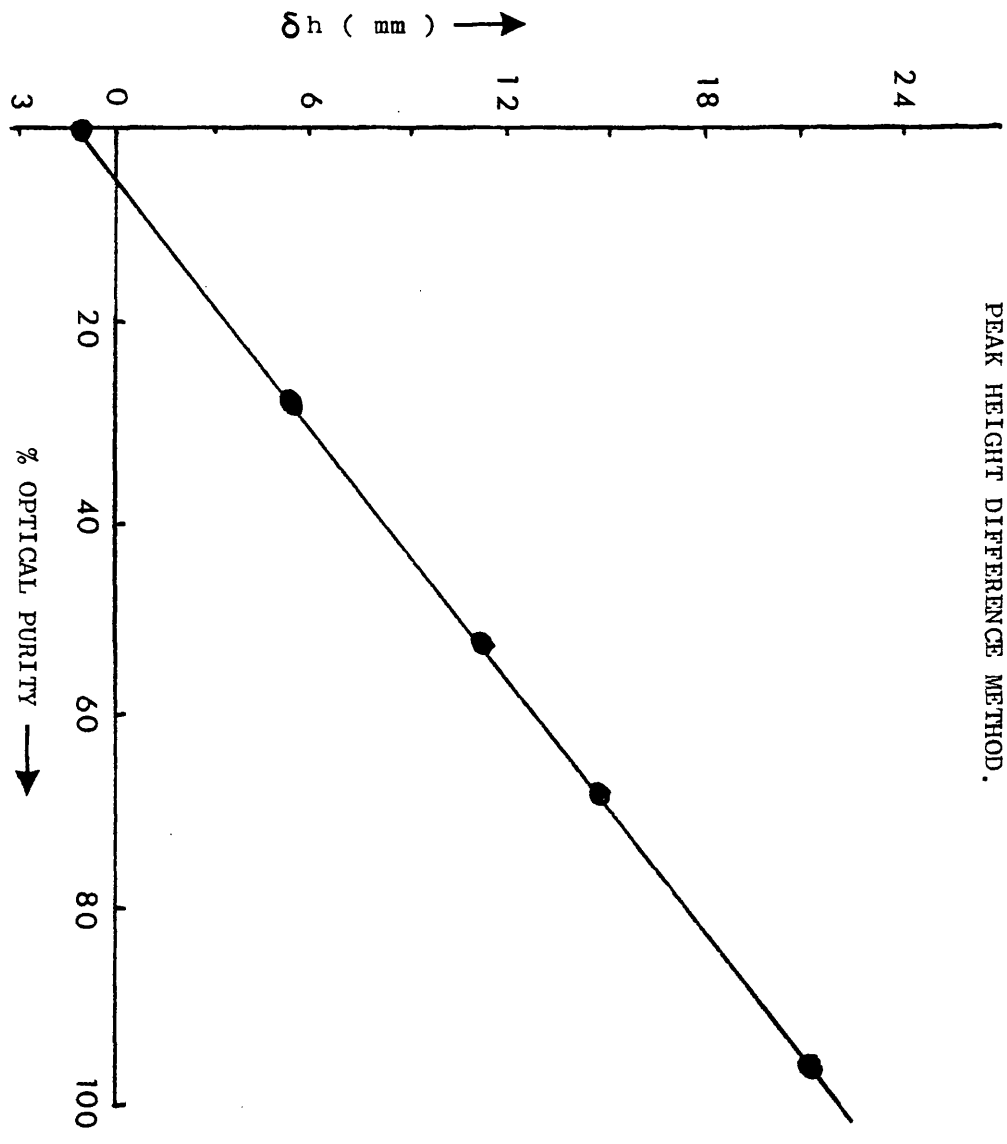


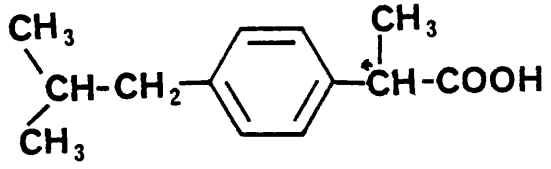
FIG. 2:23 OPTICAL PURITY DETERMINATION OF TETRAMISOLE BY PEAK HEIGHT DIFFERENCE METHOD.

The optical purity by the integration method was obtained by the use of the following formula:

$$\text{optical purity} = \frac{I' - I}{I' + I} \times 100$$

where I' and I represent the integrals for the resonance paks, with I' being the isomer of larger quantity. A plot of the δh against the optical purity is shown in figure 2:23 (page 111).

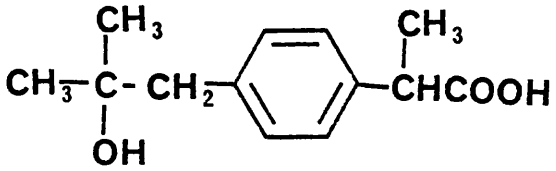
2.2.6 IBUPROFEN



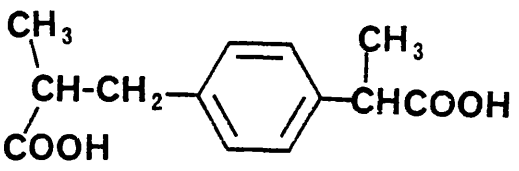
(2:13)

Ibuprofen (2-(4-isobutylphenyl)propanoic acid) (2:13) is an anti-inflammatory analgesic, and antipyretic compound used for the treatment of rheumatoid arthritis and other rheumatic diseases[137,138].

Ibuprofen consists of two enantiomorphs of equal anti-inflammatory potency. However, the principal urinary metabolites (2:14, 2:15) are dextrorotatory[138]. The resolution of ibuprofen has been effected with (-)-1-phenylethylamine[139].



(2:14)



(2:15)

The two major human metabolites are inactive in anti-inflammatory and analgesic tests, suggesting that ibuprofen is *per se* an active pharmacological agent[138]. In humans, the (R)(-)-ibuprofen is epimerized to its optical antipode (S), (+)[139].

Ibuprofen is a colourless, crystalline, stable solid of melting point 75 - 77°C. It is slightly soluble in water but readily soluble in most organic solvents. The sodium salt is readily soluble in water[137,138].

GLC methods have been utilized for the measurement of the individual enantiomers[139]. The resolution involves the formation of diastereromeric amides with either (S)-(-)-α-methylbenzylamine or (R)-(+)-α-methylbenzylamine. Determination of the enantiomeric purity by the above method is time consuming and tedious. The use of nmr with chiral shift reagents appears to be simpler and faster.

In this work, the optical purity of ibuprofen has been determined by the direct application of the chiral shift reagent $Eu(facam)_3$ in CCl_4 at a molar ratio of 0.36, using the C-C*H*$_3$ signals. The time involved in a single determination was less than 30 minutes after the preliminaries had been carried out. The base line technique, the chemical shift difference and the peak height difference methods were employed for the analysis. The optical purities of ibuprofen in 'Brufen' tablets and suspension were also determined using the calibration curves obtained from the pure samples. The results for the optical purity

Table 2:17A LIS of ibuprofen with $Eu(fod)_3$ in $CDCl_3$

[Ibuprofen] = 0.212 M

MOLAR RATIO $Eu(facam)_3$ / ibuprofen	$C(CH_3)_2$		$C-CH_3$		$-CH-$		$-CH_2-$		$-*CH$		Aromatic protons H_2 & H_6		Aromatic protons H_3 & H_5	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν
0.0	0.88	0.0	1.48	0.0	1.83	0.0	2.42	0.0	3.67	0.00	7.15	0.0	7.11	0.00
0.062	0.81	-0.07	1.70	0.22	1.79	-0.04	2.38	-0.04	3.75	0.08	7.40	0.25	7.06	-0.05
0.118	0.73	-0.15	1.98	0.50	1.69	-0.14	2.31	-0.11	4.00	0.33	7.64	0.49	7.05	-0.06
0.199	0.57	-0.31	2.38	0.90	1.57	-0.26	2.19	-0.23	4.36	0.69	7.89	0.74	6.99	-0.12
0.293	0.39	-0.49	2.81	1.33	1.39	-0.44	2.01	-0.41	4.73	1.06	8.18	1.03	6.89	-0.22
0.398	0.16	-0.72	3.26	1.78	1.19	-0.64	1.81	-0.61	5.20	1.53	8.46	1.31	6.78	-0.33

Table 2.17C LIS and ΔLIS of ibuprofen using $Eu(facam)_3$ in $CDCl_3$

$[\pm$ Ibuprofen$]$ = 0.211 M

MOLAR RATIO $Eu(facam)_3$ / I Ibuprofen	$C(CH_3)_2$		$-CH_2-$		$-*CH-$		Aromatic protons H_3		Aromatic protons H_2 & H_6 l-isomer		Aromatic protons H_2 & H_6 d-isomer			$C-CH_3$ l-isomer		$C-CH_3$ d-isomer		
	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	ΔΔν
0.0	0.88	0.0	2.42	0.0	3.67	0.0	7.11	0.0	7.15	0.0	7.15	0.0	0.0	1.48	0.0	1.48	0.0	0.00
0.115	0.76	-0.12	2.34	-0.08	3.78	0.11	7.02	-0.09	7.48	0.33	7.44	0.29	0.04	1.76	0.28	1.68	0.20	0.08
0.231	0.59	-0.29	-	-	4.18	0.51	6.96	-0.15	7.89	o0.72	7.76	0.61	0.11	2.19	0.71	2.03	0.55	0.16
0.308	0.43	-0.45	2.06	-0.30	4.43	0.76	6.89	-0.22	8.07	0.92	7.96	0.81	0.11	2.48	1.00	2.27	0.79	0.21
0.352	0.33	-0.55	1.95	-0.47	4.60	0.93	6.81	-0.30	8.18	1.03	8.05	0.90	0.13	2.63	1.15	2.39	0.91	0.24

Table 2:17B LIS of ibuprofen using $Pr(fod)_3$ in $CDCl_3$

[Ibuprofen] = 0.232 M

MOLAR RATIO $Pr(fod)_3$ / Ibuprofen	$C(CH_3)_2$ δ	$C(CH_3)_2$ Δν	$C-CH_3$ δ	$C-CH_3$ Δν	$-CH_2-$ δ	$-CH_2-$ Δν	Aromatic protons H_2 & H_6 δ	Aromatic protons H_2 & H_6 Δν	Aromatic protons H_3 & H_5 δ	Aromatic protons H_3 & H_5 Δν
0.0	0.88	0.0	1.48	0.0	2.42	0.0	7.15	0.0	7.11	0.0
0.071	0.72	0.16	0.49	0.99	2.21	0.21	6.35	0.80	6.74	0.37
0.216	0.30	0.58	-1.60	3.08	1.72	0.70	4.66	2.49	6.02	1.09
0.309	0.00	0.88	-2.92	4.40	1.38	1.04	3.59	3.56	5.56	1.55

Table 2:17D LIS of ibuprofen using $Pr(facam)_3$ in $CDCl_3$

[± Ibuprofen] = 0.235 M

MOLAR RATIO $Pr(facam)_3$ / Ibuprofen	$C(CH_3)_2$ δ	$C(CH_3)_2$ Δν	$C-CH_3$ δ	$C-CH_3$ Δν	$-CH_2-$ δ	$-CH_2-$ Δν	Aromatic protons H_2 & H_6 δ	Aromatic protons H_2 & H_6 Δν	Aromatic protons H_3 & H_5 δ	Aromatic protons H_3 & H_5 Δν
0.0	0.88	0.0	1.48	0.0	2.42	0.0	7.15	0.0	7.11	0.0
0.037	0.80	0.08	1.17	0.31	2.33	0.09	6.92	0.23	6.92	0.19
0.100	0.59	0.29	0.59	0.89	2.08	0.34	6.27	0.88	6.58	0.53
0.167	0.38	0.50	-0.15	1.63	1.85	0.57	5.67	1.48	6.32	0.79
0.227	0.18	0.70	-0.77	2.25	1.63	0.79	5.08	2.07	6.00	1.11

Table 2:18 Optical purity determination of ibuprofen

Solvent: CCl_4

Reagent: $Eu(facam)_3$

*Molar Ratio: 0.366 ± 0.002

[ibuprofen]= 0.23 M

Analytical peaks: C-CH_3

Resonance positions: l.....δ3.52 ppm

d.....δ3.14 ppm

Optical purity (%)	$1 - \frac{O.P.}{100}$	Separation (S; cm)	Chemical shift difference (δv; ppm)	Peak height difference (δh; mm)
100.0	0.00	0.00	-	32.2
75.0	0.25	0.15	0.52	22.9
47.6	0.52	0.29	0.49	15.8
23.7	0.76	0.44	0.37	8.0
0.0	1.00	0.59	0.24	0.7

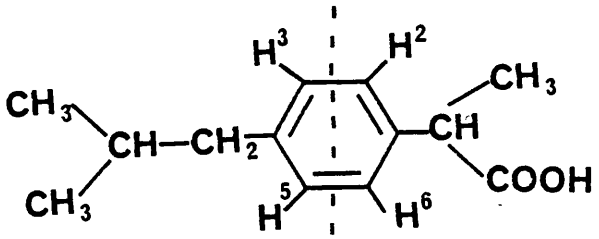
* Reagent / Ibuprofen

determination are given in table 2:18 (page 117) and in figures 2:26 and 2:27 (page 122). Results for the LIS and ΔLIS are also given in tables 2:17A - D (page 114) and in figures 2:24 and 2:25 (pages 120 and 121).

2.2.6.1 Results and Discussion

Lanthanide Induced Shift:

When $Eu(fod)_3$ was used as the shift reagent, some of the groups shifted downfield while others shifted upfield. It is interesting to note that when the molecule (ibuprofen) is divided into two parts through the benzene ring as shown below, all the protons or



Left (Upfield shift) Right (Downfield shift)

groups of protons on the left hand side show upfield shifts and all those on the right hand side show downfield shifts. The downfield shifts are larger than the upfield shifts since the site of co-ordination is on the right hand side of the molecule. Fig. 2:24A (page 120) shows the plots of the LIS against the molar ratio for the various groups of protons except for *OH* which could not be plotted on the same scale in view of the large shift.

Different results were obtained using the praseodymium analogue

$Pr(fod)_3$ (figure 2:24B, page 120). All groups showed an upfield shift. The order of the LIS, as expected was: OH > CH_3 > *CH > H_2 and H_6 > H_3 and H_5 > CH_2 > CH > $C(CH_3)_3$. Larger induced shifts were obtained with $Pr(fod)_3$ than with $Eu(fod)_3$. However, broadening occurred at much lower concentrations of $Pr(fod)_3$ than with $Eu(fod)_3$. The pattern of the graphs (fig. 2:24, page 120) was also interesting. The LIS obtained using $Pr(fod)_3$ were linear, passing through the origin. Though those obtained with $Eu(fod)_3$ were also linear, none passed through the origin. The pattern of the induced shifts obtained using the chiral shift reagents $Eu(facam)_3$ and $Pr(facam)_3$ were similar to those of their non-chiral shift reagent analogues.

At the concentrations of $Pr(facam)_3$ employed there was no separation of the isomers. The experiment was discontinued at a molar ratio of 0.227 because the resonance peaks became broad and featureless. With the $Eu(facam)_3$, differential shift was obtained. At the concentrations of $Eu(facam)_3$ employed, the C-CH_3, and the aromatic protons H_2 and H_6 gave differential shifts for the (+) and (-) isomers. Fig. 2:25 (page 121) shows plots of the C-CH_3 resonances *versus* the molar ratio, and the differential shift ($\Delta\Delta\nu$) *versus* the molar ratio. The (-) isomer gave larger LIS than the (+) isomer. Though the LIS plots of the C-CH_3 for the (-) and (+) isomers did not pass through the origin, the $\Delta\Delta\nu$ plot passed through the origin .

Optical purity determination:

The C-CH_3 signal gave sufficient separation for analytical

Fig. 2:24

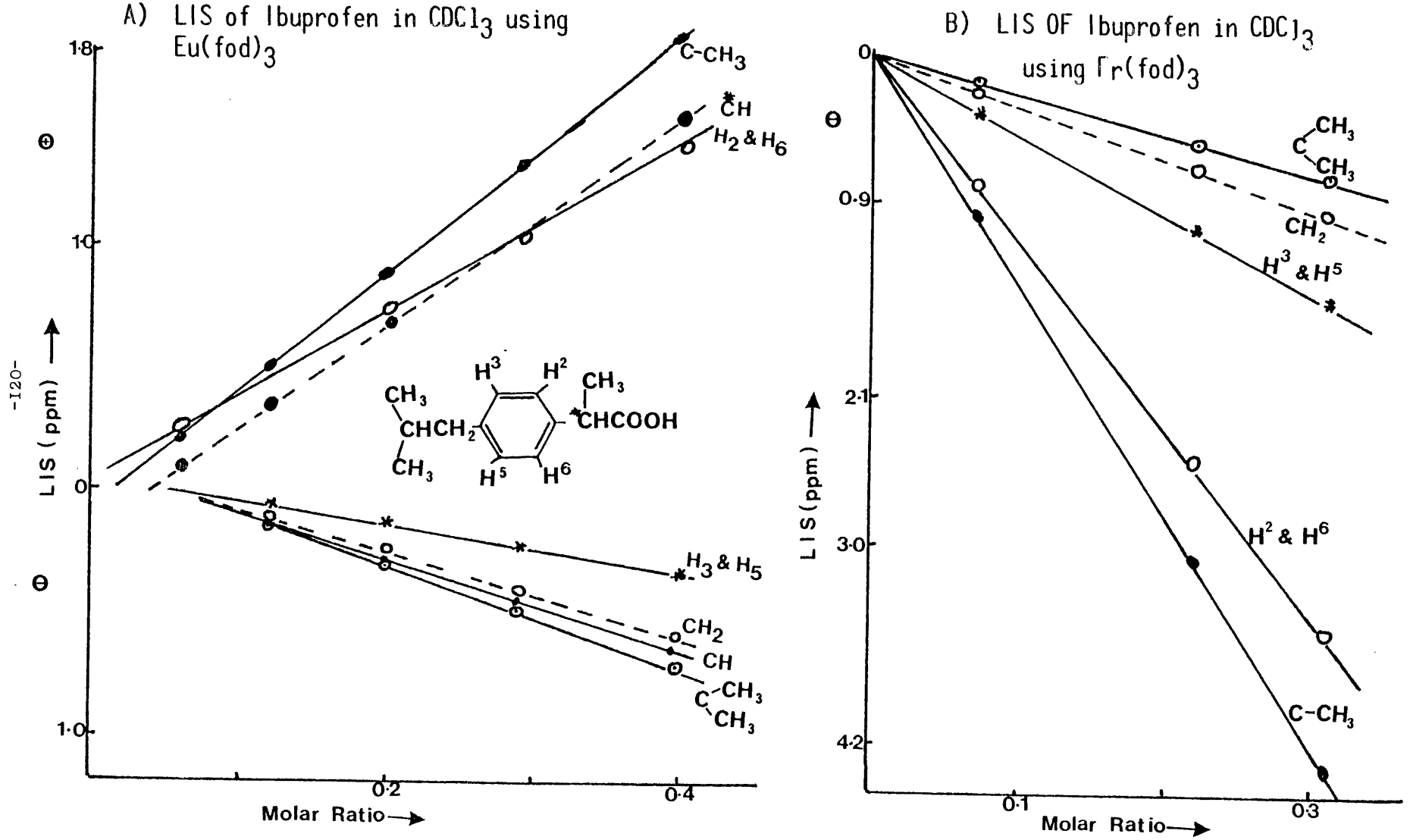

A) LIS of Ibuprofen in $CDCl_3$ using $Eu(fod)_3$
LIS (ppm)
Molar Ratio
C-CH3
CH
H2 & H6
H3 & H5
CH2
CH
C-CH3
B) LIS OF Ibuprofen in $CDCl_3$ using $Pr(fod)_3$
LIS (ppm)
Molar Ratio
CH3 C CH3
CH2
H3 & H5
H2 & H6
C-CH3

FIG. 2:25 IBUPROFEN C-CH_3 LIS & ΔLIS IN $CDCl_3$.

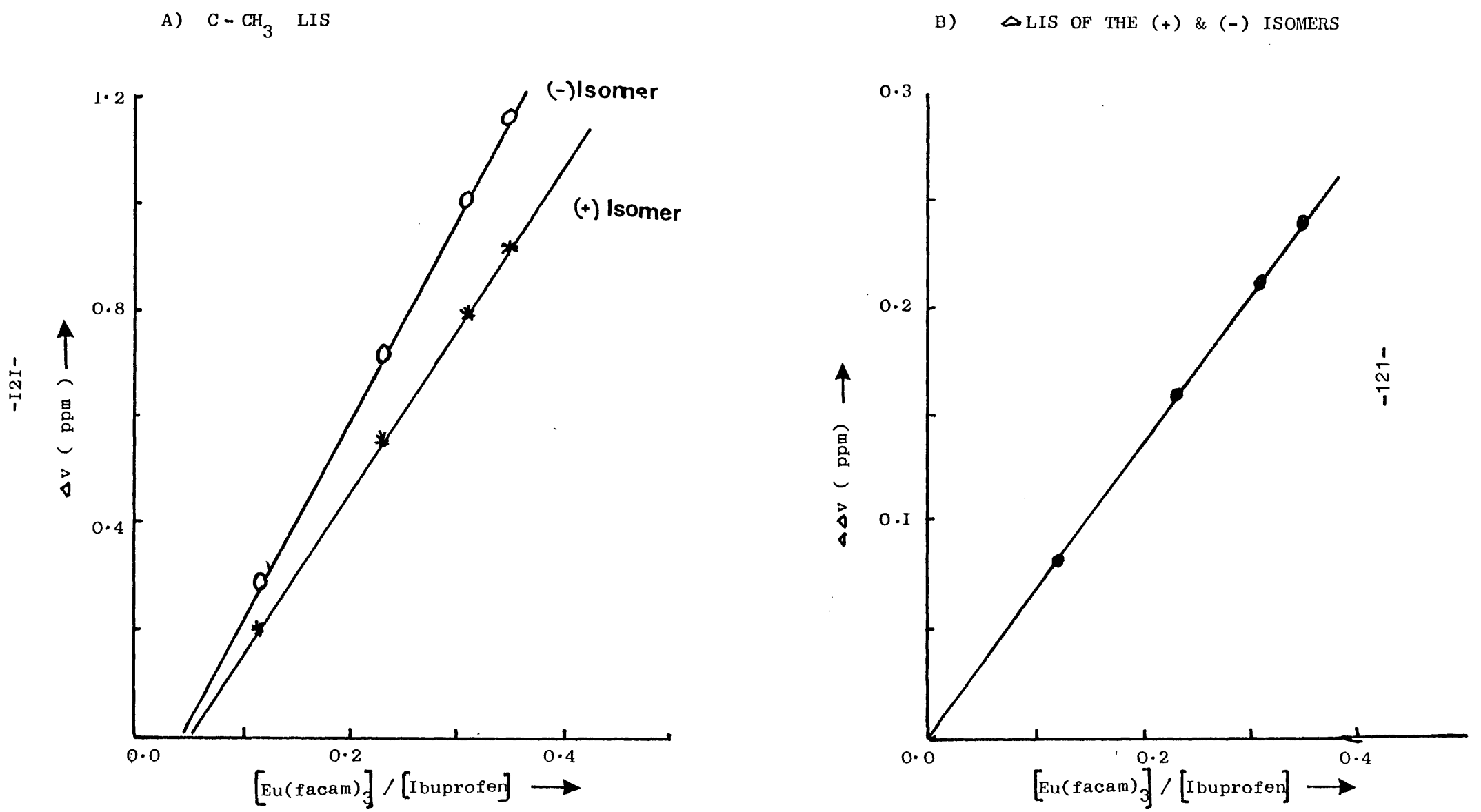


FIG. 2:26 The Base line technique for Optical purity determination of Ibuprofen

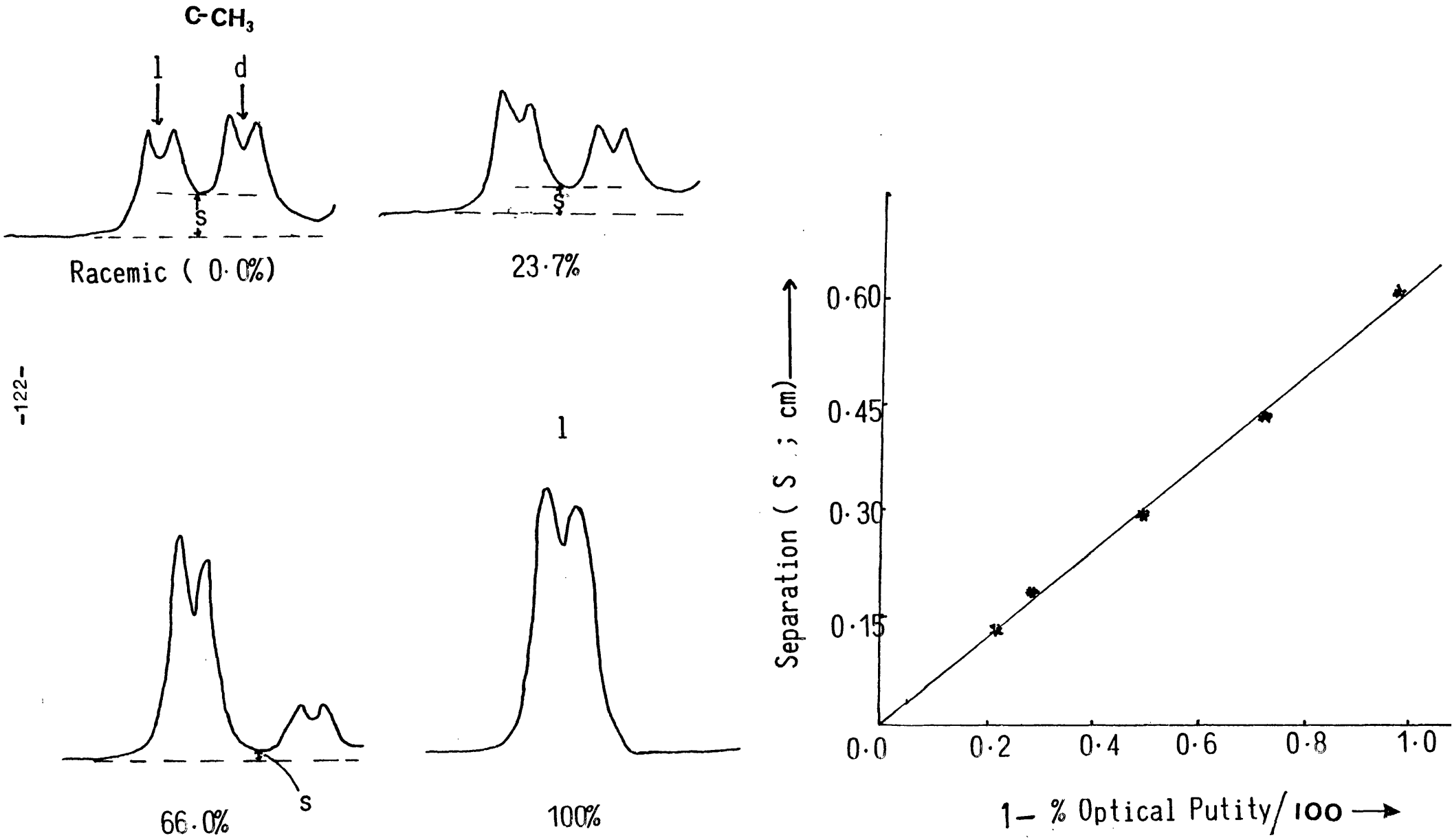
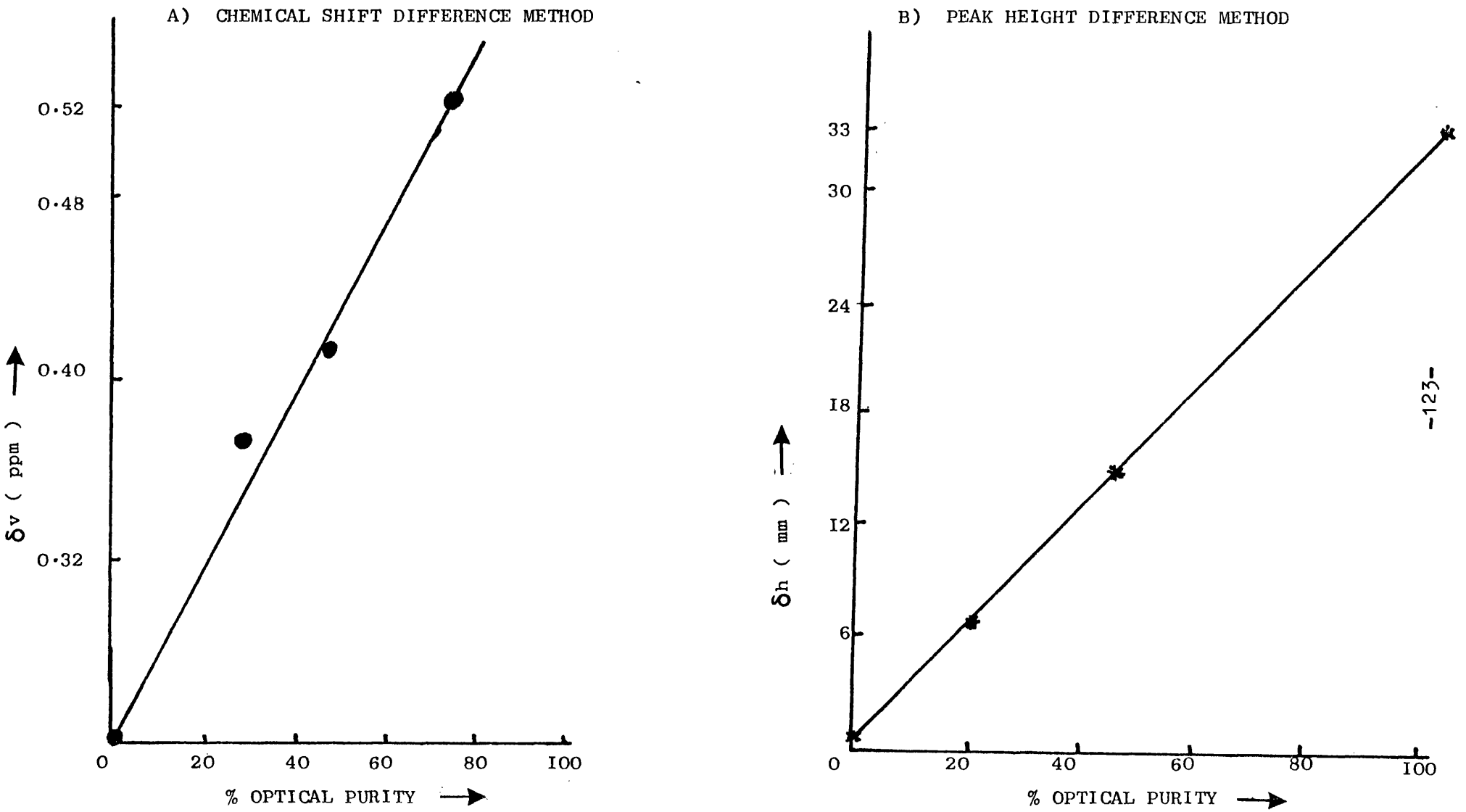


FIG. 2:27 OPTICAL PURITY DETERMINATION OF IBUPROFEN

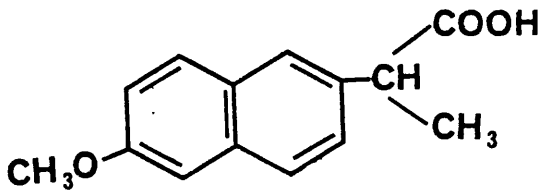


-123-

purposes. Complete separation was not achieved in view of the broadness of the peaks and integration was not feasible. Improved, though not complete, separation was achieved by changing the solvent from $CDCl_3$ to CCl_4. (For the same concentration of substrate and the same molar ratio, CCl_4 induces larger differential shifts than $CDCl_3$.)

All the new techniques - the base line, chemical shift and the peak height difference methods - gave a linear relationship when the separation (S), δv and δh were plotted against 1 - % OP/100 or % optical purity. The optical purity for the ibuprofen in the brufen tablets and suspension was found to be 0.0%.

2.2.7 NAPROXEN



(2:16)

Naproxen (2-(6-methoxy-2-naphthyl)propanoic acid) (2:16) was first developed as an anti-inflammatory agent by Harrison *et al.*[140]. It is 0.7, 5.5 and 11 times as active as indomethacin, aspirin and phenylbutazone respectively, in inhibiting the carrageenin-induced inflammation of the rat paw[140-144]. Most of its activity is due to the D(+) enantiomer[140]. In humans, naproxen is excreted

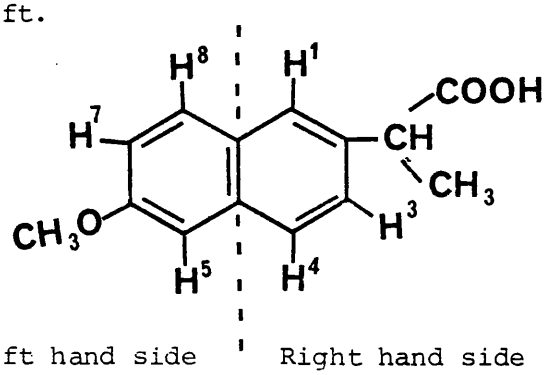
as demethylated naproxen (28%), glucuronide ester ($\simeq$ 65%) and unchanged naproxen (5 - 6%). The main route of excretion is *via* urine[144].

Like ibuprofen, the enantiomeric purity of naproxen has been determined in this work by the use of Eu(facam)$_3$ using the base line technique, the chemical shift difference and the peak height difference methods. Counting of squares under the analytical peaks (C-C*H*$_3$) was also used for the optical purity determination. The results for the optical purity determinations are given in tables 2:20A and B (page 131) and in figures 2:30 and 2:31 (pages 131 & 137). The results for the LIS are given in tables 2:19A - E and in figure 2:29 (page 135). Figure 2:28 (page 134) shows the nmr spectrum of naproxen in $CDCl_3$.

2.2.7.1 Results and Discussion

Lanthanide Induced Shift (LIS):

The pattern of the LIS was similar to that of ibuprofen. With Eu(fod)$_3$, protons or group of protons on the right hand side of the molecule (see below) gave downfield shift and those on the left hand gave upfield shift.



Left hand side Right hand side

Table 2:19A LIS of Naproxen using $Eu(fod)_3$ in $CDCl_3$

[Naproxen] = 0.25 M

*MOLAR RATIO	$C-CH_3$		OCH_3		CH		H_5 & H_7		H_4 & H_8		H_1 & H_3	
	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν	δ	Δν
0.0	1.54	0.0	3.85	0.00	3.87	0.0	7.15	0.0	7.62	0.00	7.38	0.0
0.097	1.81	0.27	3.72	-0.13	3.87	0.0	7.01	-0.14	7.58	-0.04	7.78	0.26
0.221	2.15	0.61	3.44	-0.41	4.05	0.18	6.70	-0.45	7.39	-0.23	7.99	0.61
0.354	2.49	0.95	3.08	-0.77	4.30	0.43	6.40	-0.75	7.18	-0.44	8.25	0.87
0.545	2.74	1.20	2.50	-1.35	-	-	5.83	-1.32	6.73	-0.89	8.29	0.91

* MOLAR RATIO = $Eu(fod)_3$ / Naproxen

Table 2:19B LIS of Naproxen using $Pr(fod)_3$ in $CDCl_3$

[Naproxen] = 0.25 M

MOLAR RATIO	$C\text{-}CH_3$		OCH_3		H_1		H_3	
	δ	Δv	δ	Δv	δ	Δv	δ	Δv
0.0	1.54	0.0	3.85	0.00	7.38	0.0	7.38	0.0
0.137	-0.39	1.93	3.51	0.34	6.00	1.38	5.56	1.82
0.232	-1.89	3.43	3.31	0.54	4.86	2.52	4.32	3.06
0.347	-3.32	4.86	2.99	0.86	3.60	3.78	2.81	4.57
0.474	-4.97	6.51	2.58	1.27	2.23	5.15	1.39	5.99
0.604	-6.50	8.04	2.00	1.85	1.00	6.38	-	-

*MOLAR RATIO: $Pr(fod)_3$ / Naproxen

Table 2:19C LIS and ΔLIS of Naproxen using $Eu(facam)_3$ in $CDCl_3$

(±) Naproxen = 0.29 M

MOLAR RATIO $\frac{Eu(facam)_3}{\pm \text{ Naproxen}}$	$O\text{-}CH_3$		CH		$C\text{-}CH_3$				
					l-isomer		d-isomer		
	δ	Δν	δ	Δν	δ	Δν	δ	Δν	ΔΔν
0.0	3.85	0.0	3.85	0.00	1.54	0.0	1.54	0.0	0.0
0.118	3.64	-0.21	3.85	0.00	1.64	0.10	1.60	0.06	0.04
0.226	3.36	-0.49	4.00	0.15	1.88	0.34	1.76	0.22	0.12
0.304	3.13	-0.72	4.20	0.35	2.03	0.49	1.84	0.30	0.19
0.368	2.92	-0.93	4.33	0.48	2.15	0.61	1.90	0.36	0.25
0.433	2,62	-1.23	4.40	0.55	2.18	0.64	1.90	0.36	0.28
0.572	1.97	-1.88	-	-	*	-	*	-	-

- broad and featureless

* merged with OCH_3 signal.

Table 2:19D LIS of Naproxen using $Pr(facam)_3$ in $CDCl_3$

$[(\pm)$ Naproxen$]$ = 0.29 M

MOLAR RATIO $\frac{Pr(facam)_3}{Naproxen}$	$C\text{-}CH_3$		CH		OCH_3	
	δ	Δν	δ	Δν	δ	Δν
0.0	1.54	0.00	3.85	0.00	3.85	0.00
0.073	0.87	0.67	3.27	0.58	3.66	0.19
0.170	-0.14	1.68	+	-	3.39	0.46
0.297	-1.30	2.84	+	-	2.99	0.87
0.477	-2.86	4.40	+	-	2.40	1.45
0.591	-3.56	5.10	+	-	1.89	1.96

+ broad and featureless

Table 2:19E LIS of Naproxen using $Eu(facam)_3$ in $CCl_4/CDCl_3$ (1:1)

[Naproxen] = 0.29 M

MOLAR RATIO $\frac{Eu(facam)_3}{Naproxen}$	OCH_3		CH		$C\text{-}CH_3$				
					l-isomer		d-isomer		
	δ	Δν	δ	Δν	δ	Δν	δ	Δν	ΔΔν
0.000	3.85	0.00	3.85	0.00	1.54	0.00	1.54	0.00	0.00
0.185	3.52	-0.33	3.94	0.09	1.91	0.37	1.76	0.22	0.15
0.334	3.31	-0.54	4.60	0.75	2.50	0.96	2.25	0.71	0.25
0.426	3.10	-0.75	5.00	1.15	2.80	1.26	2.20	0.96	0.30
0.577	2.73	-1.12	+	-	+	-			
0.786	2.20	-1.65	+	-	+	-			
0.949	1.65	-2.20	+	-	+	-			

Table 2:20 Optical purity determination of Naproxen

Solvent: $CDCl_3/CCl_4$ (3:4)

Reagent: $Eu(facam)_3$

*Molar ratio: 0.355 ± 0.002

Analytical peaks: C-CH_3

Resonance positions: l .... δ2.30 - 2.60 ppm
d .... δ1.80 - 2.10 ppm

Naproxen = 0.29 M

A). By the new techniques

Optical purity (%)	Separation (S; cm)	$1 - \frac{O.P.}{100}$	Peak height difference (l-d) (mm)	Chemical shift difference (δv) (ppm)
0.0	1.61	1.00	0.3	0.29
24.4	1.39	0.76	12.8	0.32
49.0	1.05	0.51	23.0	0.37
70.1	0.79	0.30	33.5	0.37
100.0	0.40	0.00	49.1	-

B). By counting of squares

Actual optical purity (%)	No. of squares l-C-CH_3 resonance peak	d-C-CH_3 resonance peak	Optical purity found (%)
0.0	48	49	1.03
24.4	21	14	20.00
49.0	27	9	50.00
70.1	27	5	68.75

* Reagent / Naproxen

At low molar ratios the downfield shifts were larger than the upfield shifts. At higher reagent concentrations the upfield shifts became larger than the downfield shifts. It appears that at low $Eu(fod)_3$ concentration, the co-ordination is mostly at the *O*H. At higher reagent concentration co-ordination appears to be more favourable at the methoxy.

With the $Pr(fod)_3$ all the shifts were upfield, with the C-C*H*$_3$ group giving the largest shifts. The LIS results indicate that at molar ratios lower than 1.0 the co-ordination is mostly at the *O*H.

<u>ΔΔν</u>:

The pattern of the LIS with $Eu(facam)_3$ was slightly different from that of $Eu(fod)_3$ in that the O*CH*$_3$ gave the largest LIS even at low reagent concentrations. Differential shift was obtained for the C-C*H*$_3$ group and the plots are shown in figure 2:29 (page 135). Though the O*CH*$_3$ gave the largest LIS, no differential shift was obtained at the concentrations of the $Eu(facam)_3$ employed. The O*CH*$_3$ resonance peak remained sharp almost throughout the experiment whereas the C*H* and C-C*H*$_3$ became broad with increase in reagent concentration.

With the $Pr(facam)_3$, no differential shift was obtained at the concentration of reagent used. Apart from the O*CH*$_3$ resonance all other signals became broad, particularly those of C-C*H*$_3$ and C*H*.

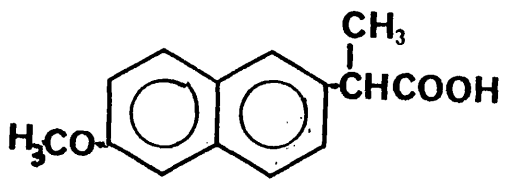
The differential shift obtained for the C-C*H*$_3$ resonance peaks

using $Eu(facam)_3$ and $CDCl_3$ was not of sufficient magnitude to allow integration. It was considered that a change of solvent from $CDCl_3$ to CCl_4 might give a larger differential shift, to allow accurate integration. Unfortunately, solubility in CCl_4 was very poor and a 1:1 mixture of $CDCl_3/CCl_4$ had to be used. This gave larger differential shifts than $CDCl_3$ alone (*cf.* tables 2:19C and E). However, complete separation was impossible since the OCH_3 resonance peak coincided with the $C\text{-}CH_3$ resonance peak. It was anticipated that at higher $Eu(facam)_3$ / (±)-Naproxen molar ratios, the OCH_3 resonance would separate from that of $C\text{-}CH_3$ to allow the $C\text{-}CH_3$ resonance peaks to be integrated, but this was not observed. A slight modification of the solvent mixture $CDCl_3/CCl_4$ (3:4) gave better separation (though not complete) and this was employed for the optical purity determination.

Optical purity determination:

Four analytical procedures were adopted: (1) the base line technique, (ii) the chemical shift difference method (iii) the peak height difference method and (iv) counting of squares. With the new techniques a linear relationship was obtained when the various parameters were plotted against (1 - OP/100) for the base line technique, or optical purity for the chemical shift difference and the peak height difference methods. The linearity of the graphs (figures 2:30 and 2:31, page 136) is an indication of the accuracy of the techniques. Table 2:20B (page 131) shows the results by counting of squares.

FIG. 2:28 NAPROXEN (0·2M) IN $CDCl_3$

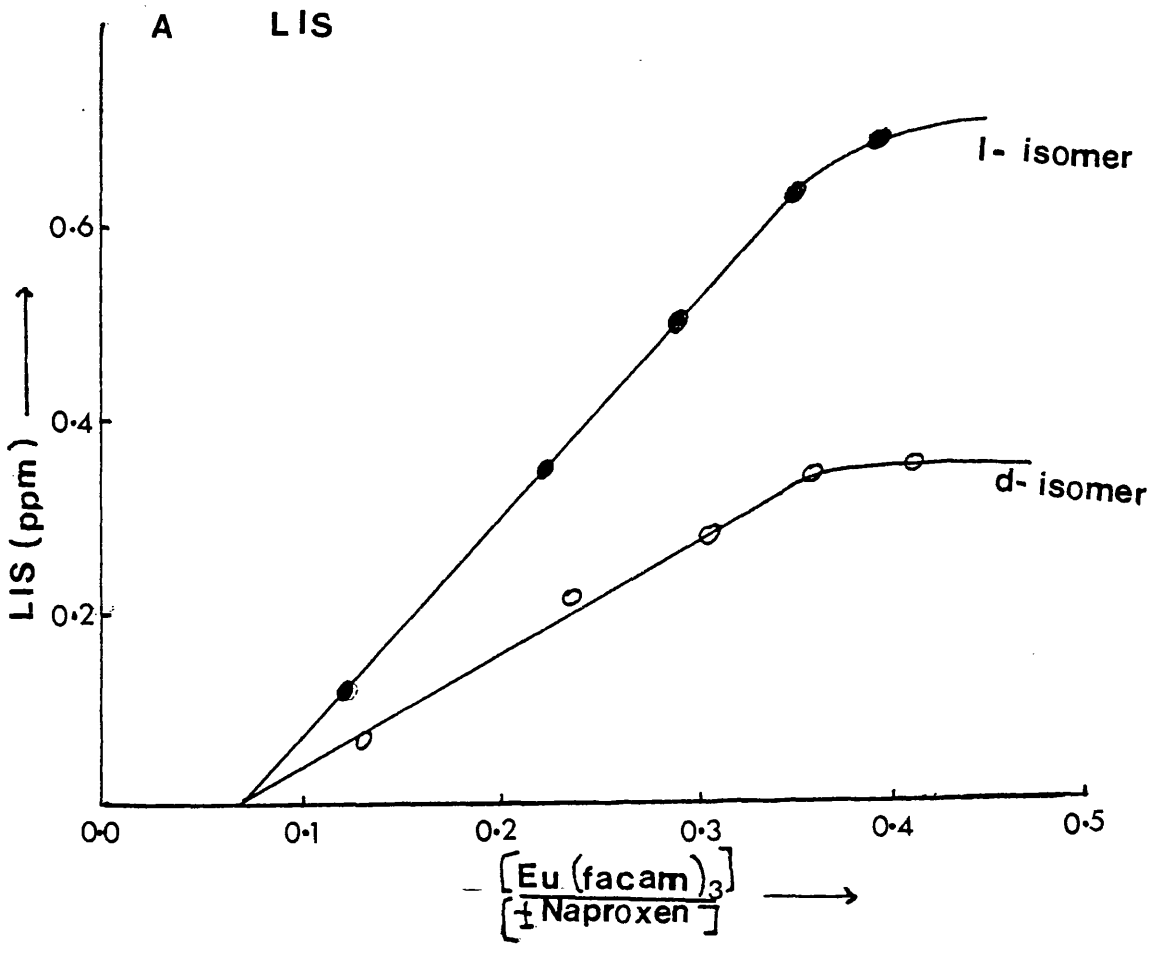


C-CH_3

TMS

8 7 6 5 4 3 2 1 0 ppm

FIG.2:29 Naproxen C-CH_3 LIS & ΔLIS



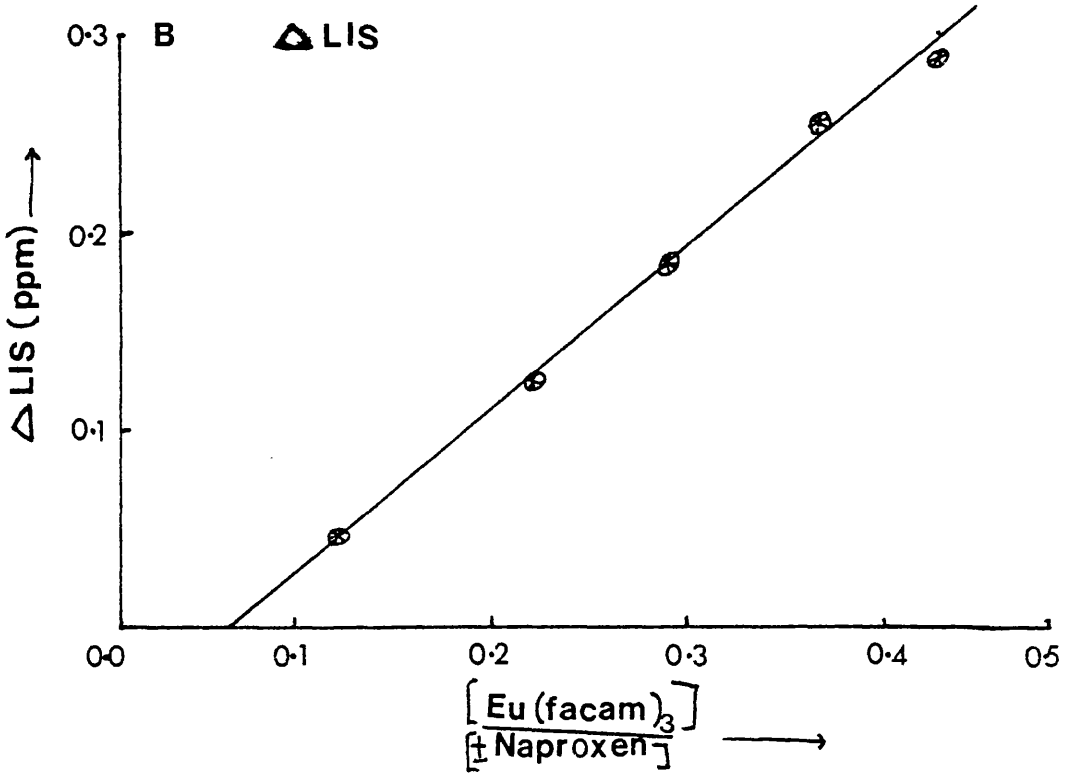
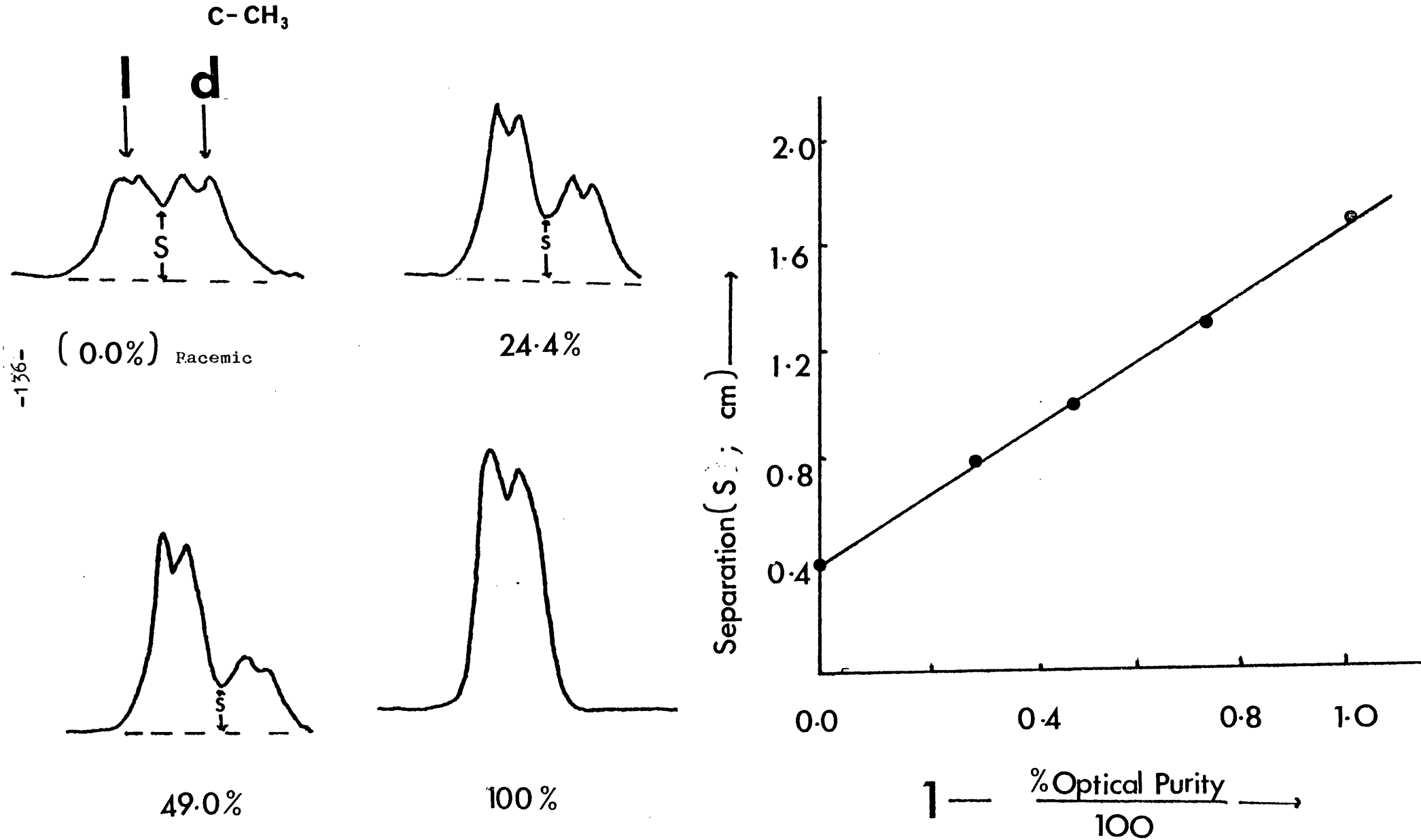


FIG. 2:30 The Base line technique for Optical purity determination of Naproxen



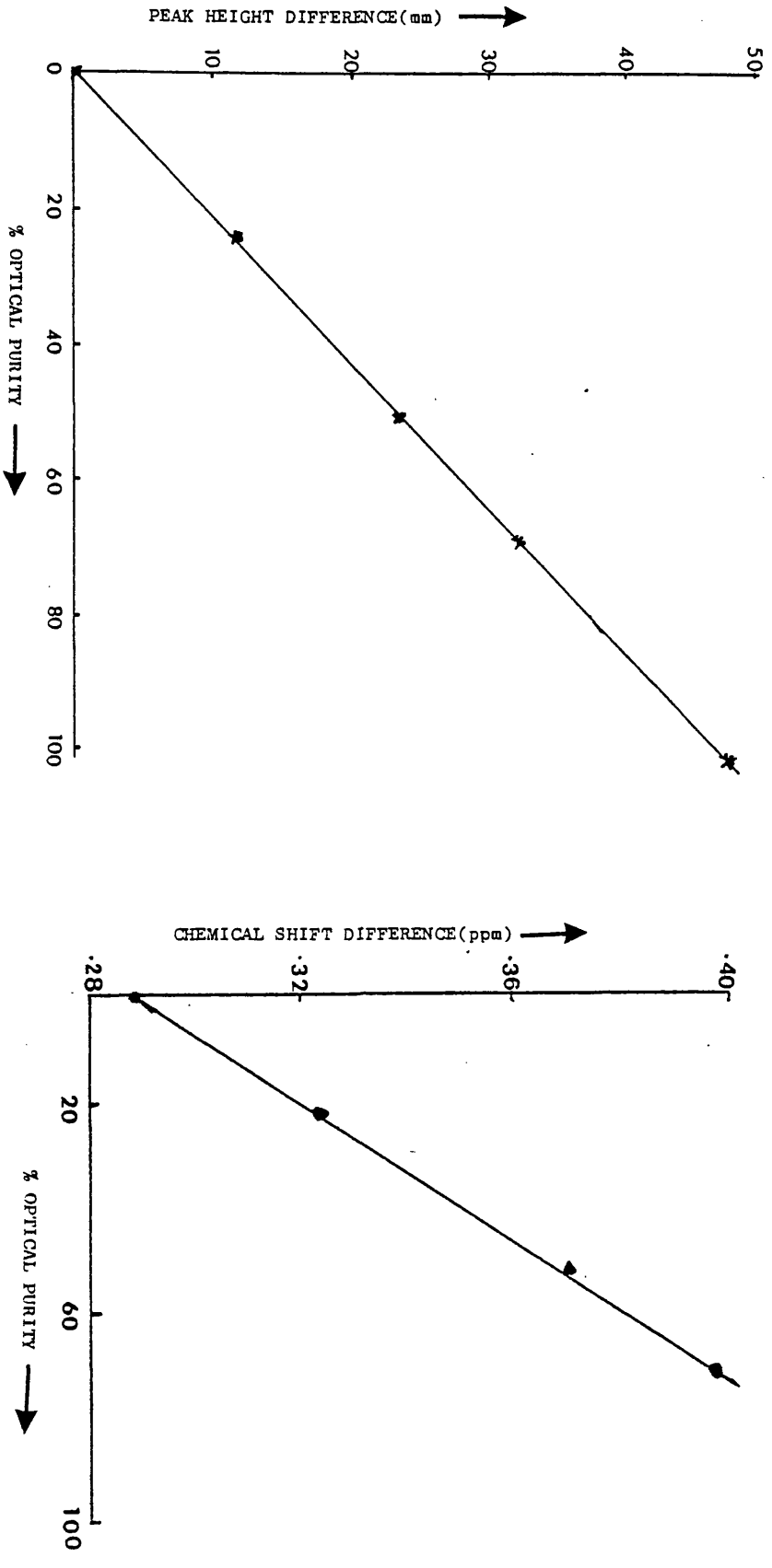
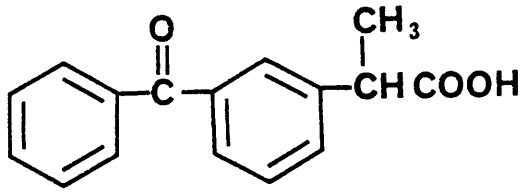


FIG.2:31 THE OPTICAL PURITY DETERMINATION OF NAPROXEN BY THE PEAK HEIGHT DIFFERENCE & THE CHEMICAL SHIFT DIFFERENCE METHODS.

2.2.8 KETOPROFEN



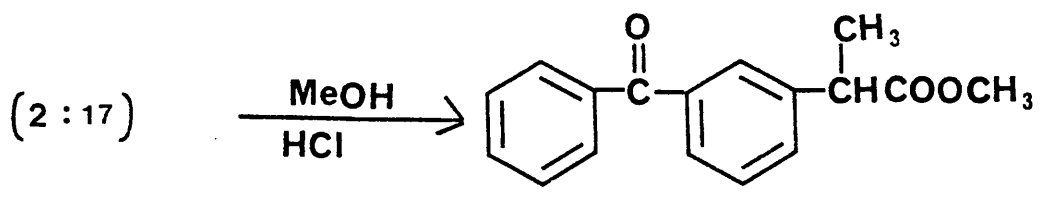
(2:17)

Ketoprofen 2-(3-benzoylphenyl)propanoic acid (2:17) is a non-steroidal anti-inflammmatory agent with a comparable therapeutic efficacy with indomethacin when given in equal dosage[145]. It also has powerful analgesic and anti-bradykinin activities[150].

The optical purity of ketoprofen has been determined from a study of its methyl ester (2:18) using $Eu(facam)_3$ in CCl_4. The base line technique and the chemical shift difference method were employed for the analysis.

2.2.8.1 Results and Discussion

Addition of a shift reagent to ketoprofen resulted in a broad signal for the C-CH_3 group. In view of this, the methyl ester derivative was used for the analysis. The equation for the methyl ester synthesis is given in Scheme 2:3 .



(2:18)

Scheme 2:3

Table 2:21 Optical purity determination of ketoprofen using the methyl ester derivative.

Solvent: CCl_4

Reagent: $Eu(facam)_3$

*Molar Ratio: 0.406

Analytical peak: C-CH_3 at δ1.9 - δ2.15 ppm.

Substrate - ketoprofen methyl ester.

Optical purity (%)	Base line technique $1 - \frac{\%\ OP}{100}$	Separation (S; mm)	Chemical shift difference (δv; Hz)
Racemic (0.0)	1.0	9.0	5.0
20.0	0.80	7.2	6.2
45.0	0.55	4.8	7.8
70.0	0.30	2.7	9.5
78.0	0.22	1.9	9.9

* Reagent / Substrate

FIG. 2.32 A) Ketoprofenmethyl ester (0·30M) in CCl_4

B) (A) in the presence of $Eu(facam)_3$ at a molar ratio ($Eu(facam)_3$ / Ketoprofenmethyl ester) of 0·406.

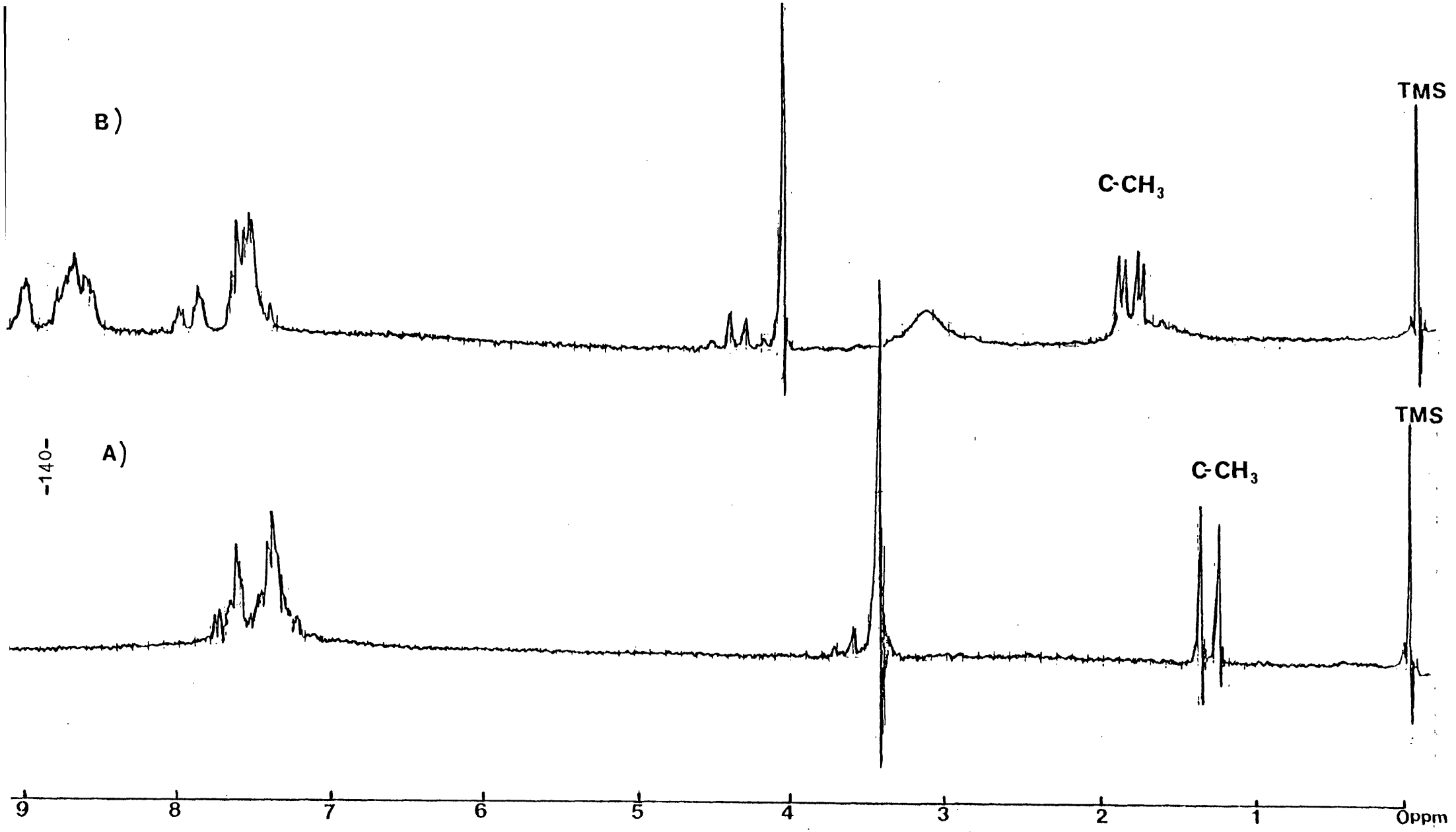

B)
TMS
C-CH3
A)
TMS
C-CH3
-140-
9
8
7
6
5
4
3
2
1
0ppm

FIG.2 : 33 Ketoprofenmethyl ester C- CH_3 signal in the presence of $Eu(facam)_3$ at different scales

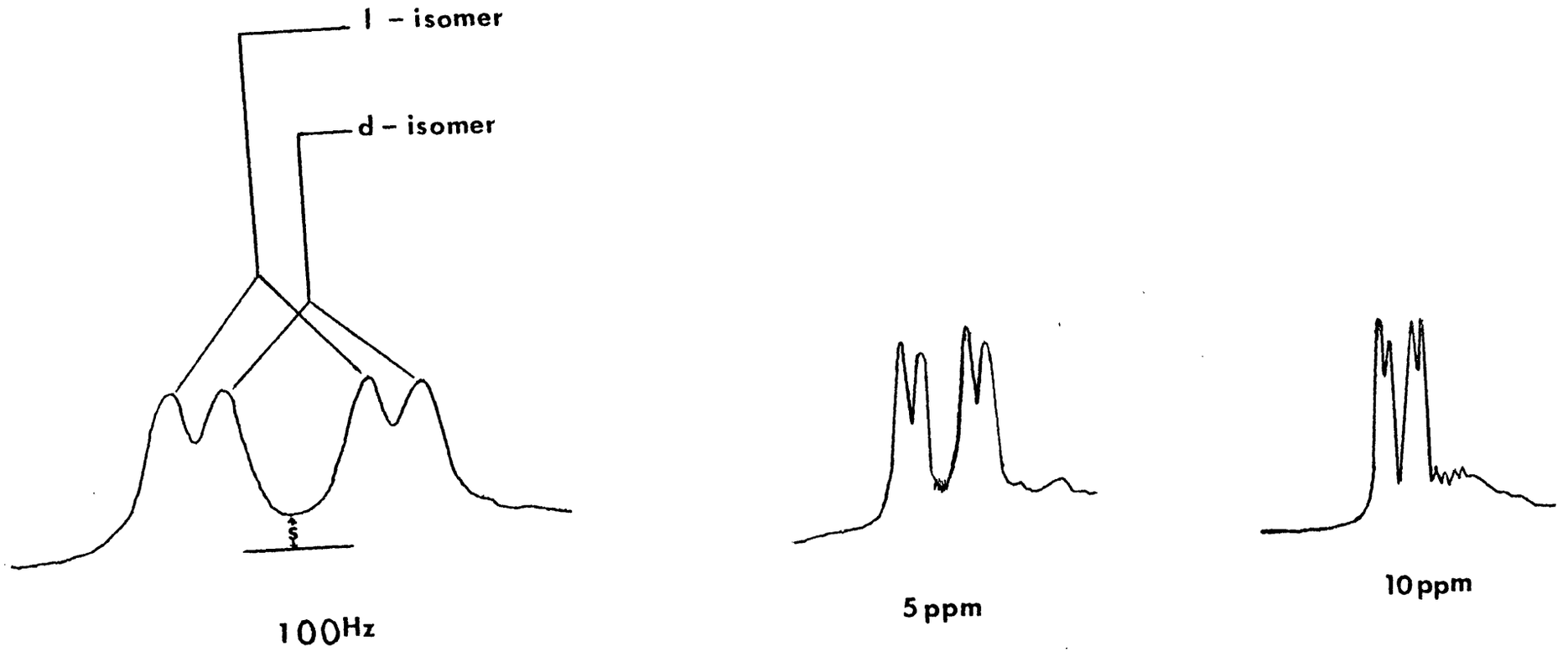
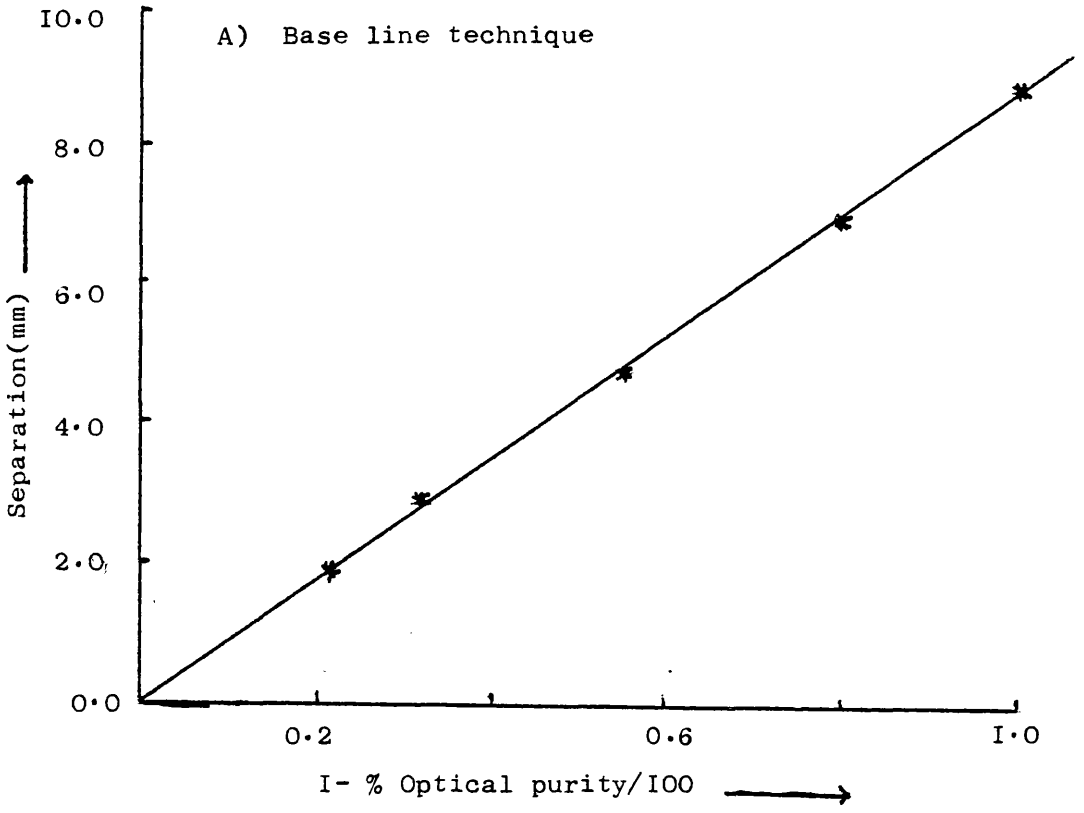
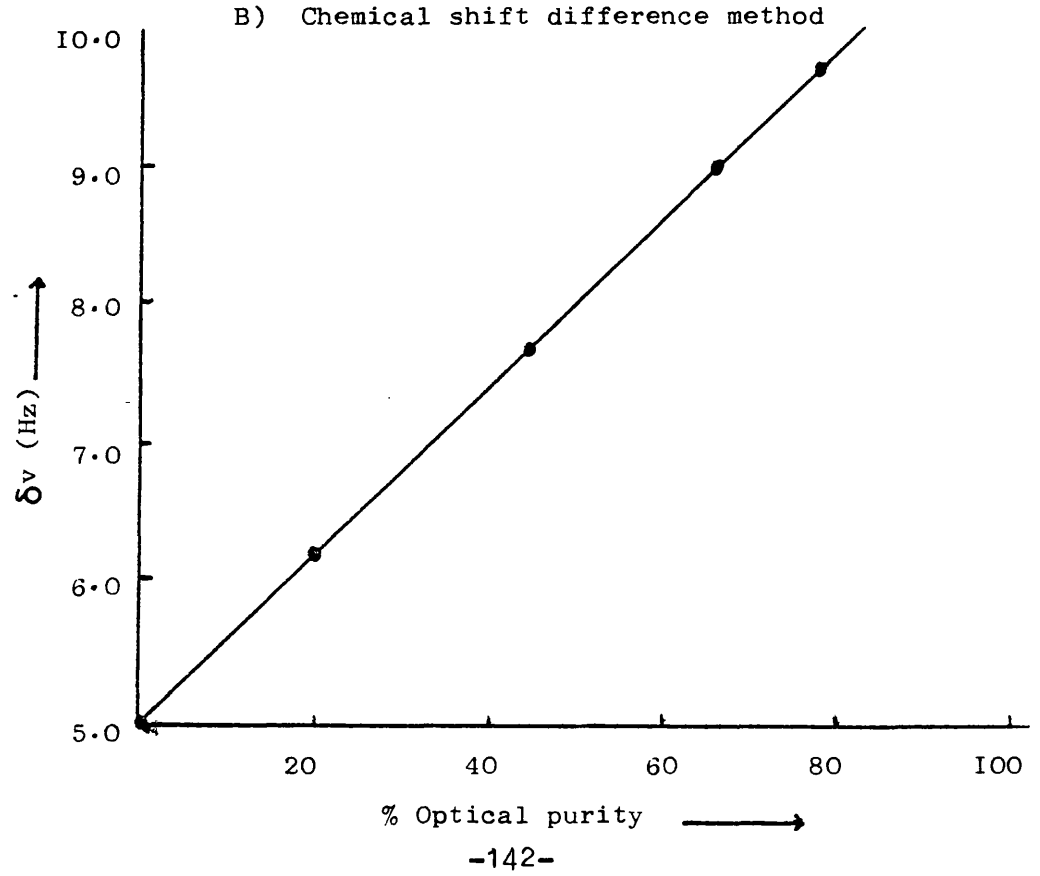


FIG. 2:34 Optical purity determination of Ketoprofen

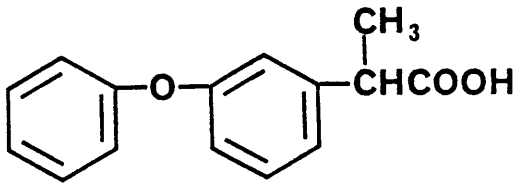




It was anticipated that with (2:18), the $COOCH_3$ signal, being a singlet, would be convenient for the analysis. Unfortunately, and surprisingly, it did not show any differential shift with $Eu(facam)_3$ However, the $C-CH_3$ signal gave a small differential shift (fig. 2:32, page 140). As with most doublets, complete separation of the $C-CH_3$ signal was not achieved. However, using scale expansion (100Hz), the base line technique and the chemical shift difference method were used for the optical purity determination. Figure 2:33 (page 141) shows the $C-CH_3$ signals (in the presence of $Eu(facam)_3$) at different scales.

Both the base line technique and the chemical shift difference method gave a linear relationship with the appropriate parameters. The results are shown in figure 2:34 (page 142) and in table 2:21.

2.2.9 FENOPROFEN



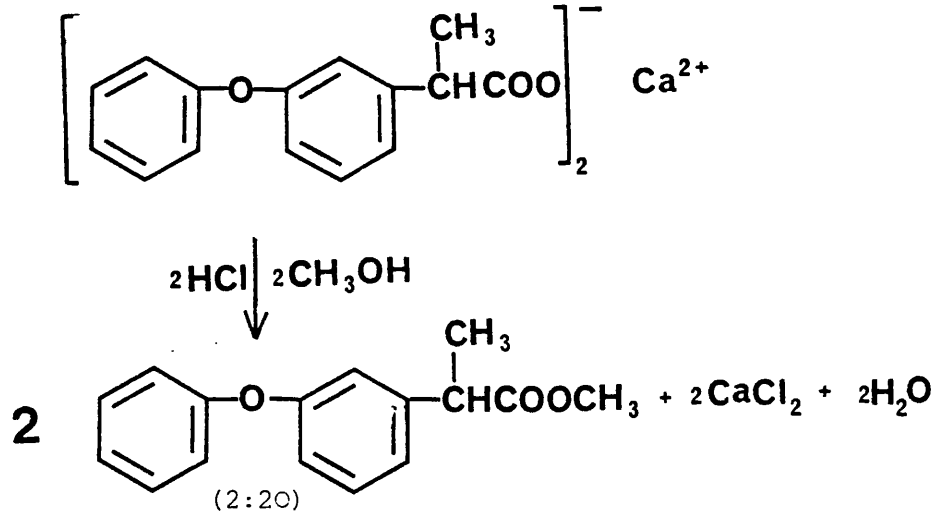
(2:19)

Fenoprofen (2-(3-phenoxyphenyl)propanoic acid) (2:19) is a viscous oil b.p. / 0.11 = 168 - 171°C, with a pKa of 7.3. It is normally used in salt form, generally the calcium salt as the dihydrate ($C_{30}H_{26}CaO_6.2H_2O$).

Fenoprofen is a non-steroidal, anti-inflammatatory, analgesic

and antipyretic agent[146-148]. It is safe and effective in the treatment of rheumatoid arthritis. It has been found effective in reducing uterine contractility without serious maternal or foetal side-effects and shows promise as a clinically effective agent for the control of premature labour[149]. Fenoprofen (1%) has a comparable efficacy with dexamethasone (0.1%) after topical application to rabbit eyes[151].

In this work the optical purity of fenoprofen has been determined using the methyl ester derivative (2:20). The calcium salt was neither soluble in water nor in non polar organic solvents. Regeneration of the acid (fenoprofen) from the calcium salt proved difficult. It was therefore necessary to use the methyl ester for the analysis (Scheme 2:4). The optical purity determination was



Scheme 2:4

performed in CCl_4 using $Eu(facam)_3$ as the shift reagent. The $COOCH_3$ signal was employed for the analysis.

2.2.9.1 Results and Discussion

The results for the ΔLIS are shown in figure 2:36 (page 148)

Table 2:22A LIS and ΔLIS of fenoprofen methyl ester in $CDCl_3$

[Fenoprofen-methyl ester] = 0.22M

MOLAR RATIO $Eu(facam)_3$ / FME	$COOCH_3$ signal					$C-CH_3$ signal	
	l-isomer		d-isomer				
	δ	Δν	δ	Δν	ΔΔν	δ	Δν
0.0	3.62	0.0	3.62	0.0	0.0	1.47	0.0
0.276	3.80	0.18	3.80	0.18	0.0	1.66	0.19
0.669	4.11	0.49	4.11	0.49	0.01	1.82	0.35
0.858	4.19	0.57	4.18	0.56	0.01	1.88	0.41
1.270	4.56	0.94	4.54	0.92	0.02	2.40	0.93

Table 2:22B LIS and ΔLIS of fenoprofen methyl ester in C_6D_6

*FME = 0.171 M

MOLAR RATIO $Eu(facam)_3$ / FME	$COOCH_3$					$C-CH_3$				
	l-isomer		d-isomer			l-isomer		d-isomer		
	δ	Δν	δ	Δν	ΔΔν	δ	Δν	δ	Δν	ΔΔν
0.0	3.24	0.0	3.24	0.0	0.0	1.32	0.0	1.32	0.0	0.0
0.386	3.93	0.69	3.92	0.68	0.01	1.85	0.53	1.84	0.52	0.01
0.723	4.26	1.02	4.24	1.00	0.03	2.08	0.76	2.06	0.74	0.02
0.946	4.42	1.18	4.39	1.15	0.04	2.13	0.81	2.10	0.78	0.03
1.134	4.56	1.32	4.52	1.28	0.04	2.30	0.98	2.26	0.94	0.04
1.555	4.72	1.48	4.68	1.44	0.04	2.49	1.17	2.45	1.13	0.04
1.884	4.91	1.67	4.87	1.63	0.04	2.52	1.20	2.48	1.16	0.04

* FME = Fenoprofen methyl ester.

Table 2:22C ΔLIS of OCH_3 and $C\text{-}CH_3$ of fenoprofen methyl ester in CCl_4

$[FME]$ = 0.215 M

MOLAR RATIO $\frac{Eu(facam)_3}{FME}$	OCH_3 ΔΔv (ppm)	$C\text{-}CH_3$ ΔΔv (ppm)
0.0	0.0	0.0
0.274	0.02	0.01
0.577	0.04	0.03
0.746	0.06	0.04
0.919	0.07	0.07

Table 2:23 Optical purity determination of fenoprofen using the methyl ester derivative

Optical purity (OP; %)	$1 - \frac{\%\ OP}{100}$	Separation (S; mm)	Peak height difference (1-d; mm)	Chemical shift difference (δv; ppm)
0.0	1.00	18.0	0.4	0.06
20.0	0.80	14.5	2.4	0.09
55.0	0.45	8.6	5.7	0.14
80.0	0.20	4.6	8.2	0.17

Solvent: CCl_4

Reagent: $Eu(facam)_3$

$[FME]$ 0.215 M

*Molar Ratio: 0.919

Analytical peaks: $COOCH_3$ at δ5.2 -δ5.3 ppm

* Reagent / FME

FIG. 2:35 A); Fenoprofenmethyl ester in CCl_4

B); (A) in the presence of $Eu(facam)_3$ at molar ratio (Fenoprofenmethyl ester/$Eu(facam)_3$) of 0·92

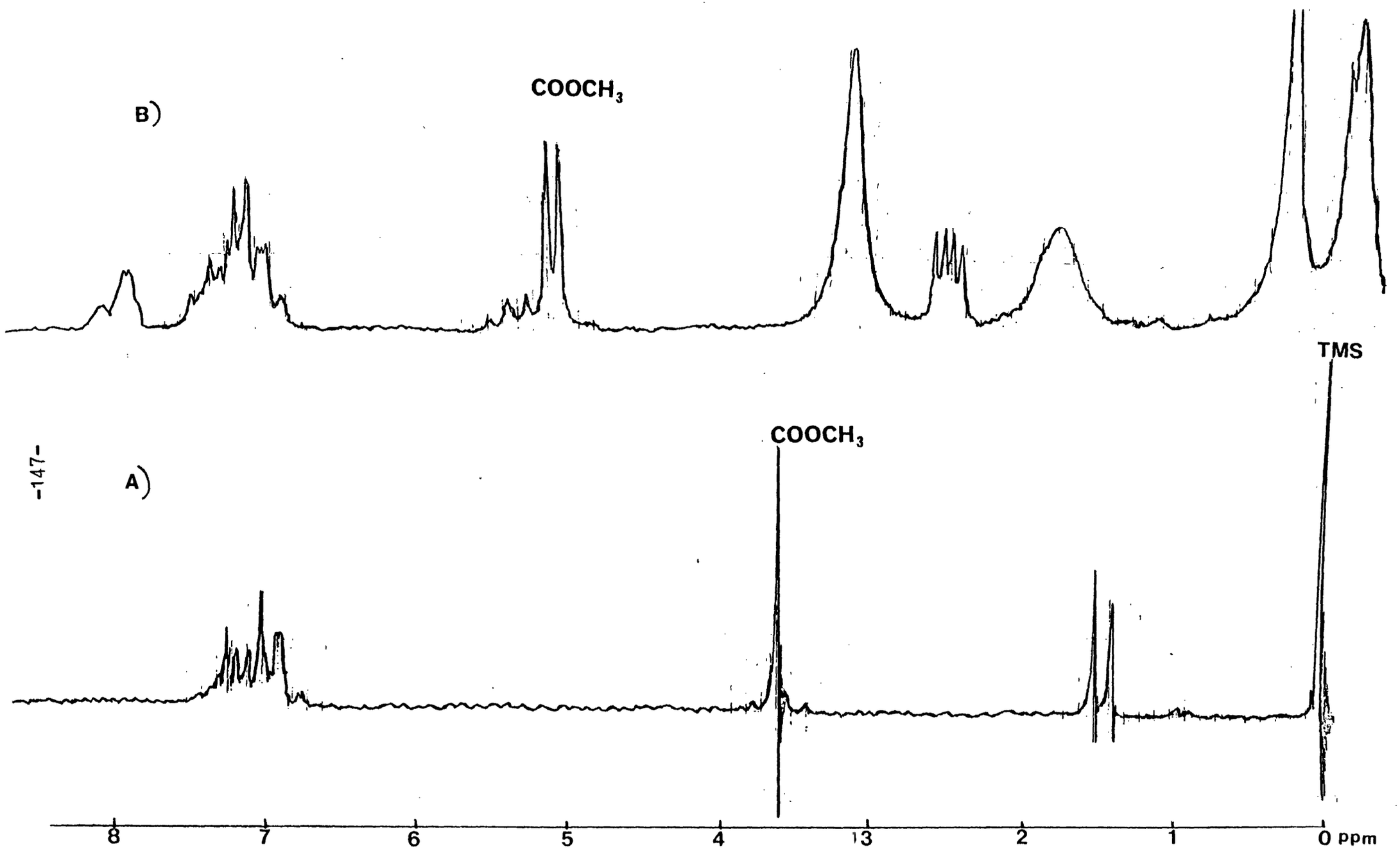
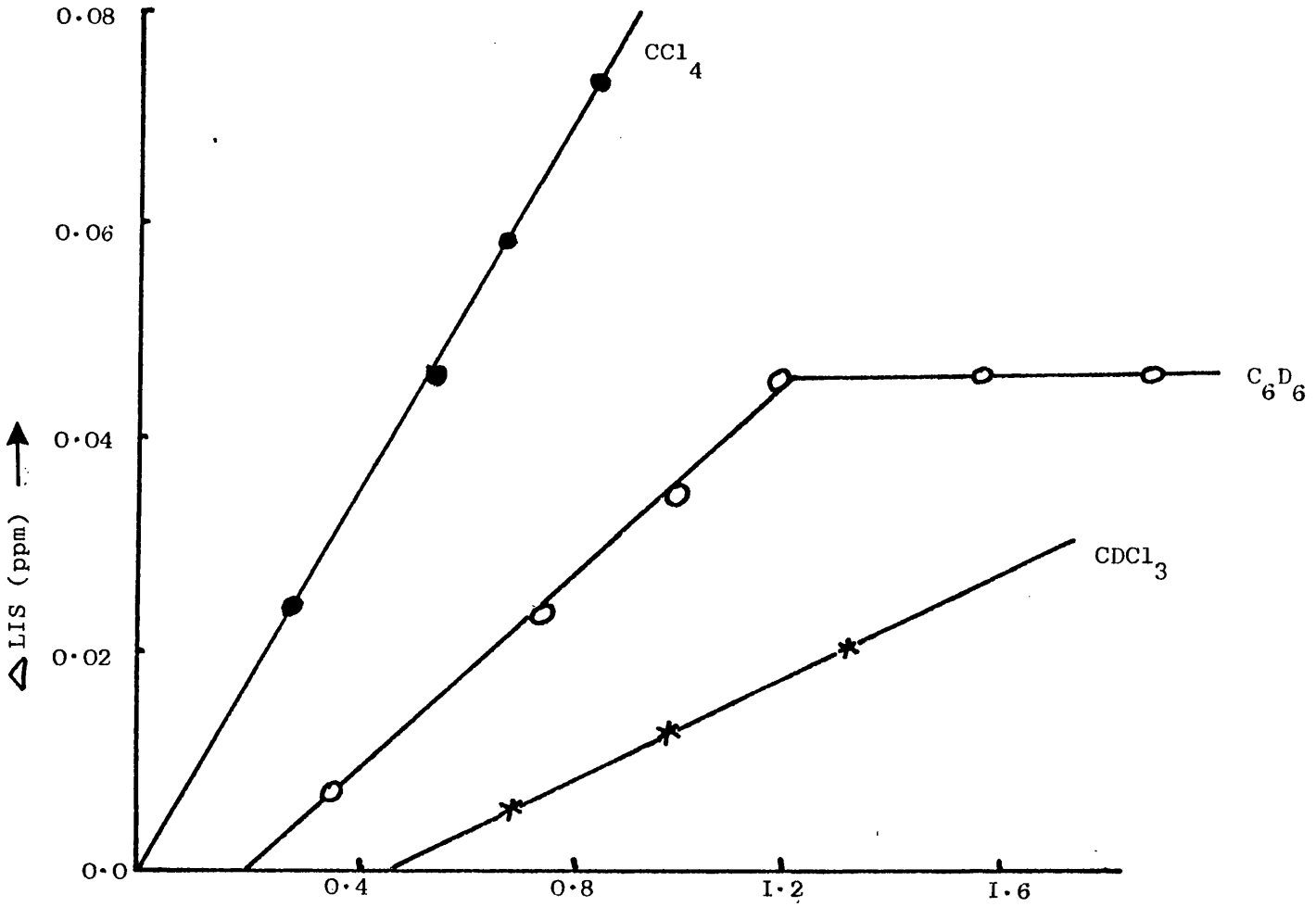

B)
COOCH3
TMS
A)
COOCH3
8
7
6
5
4
3
2
1
0 ppm

FIG. 2:36 Fenoprofen methyl ester ΔLIS of the $COOCH_3$ signal in different solvents.



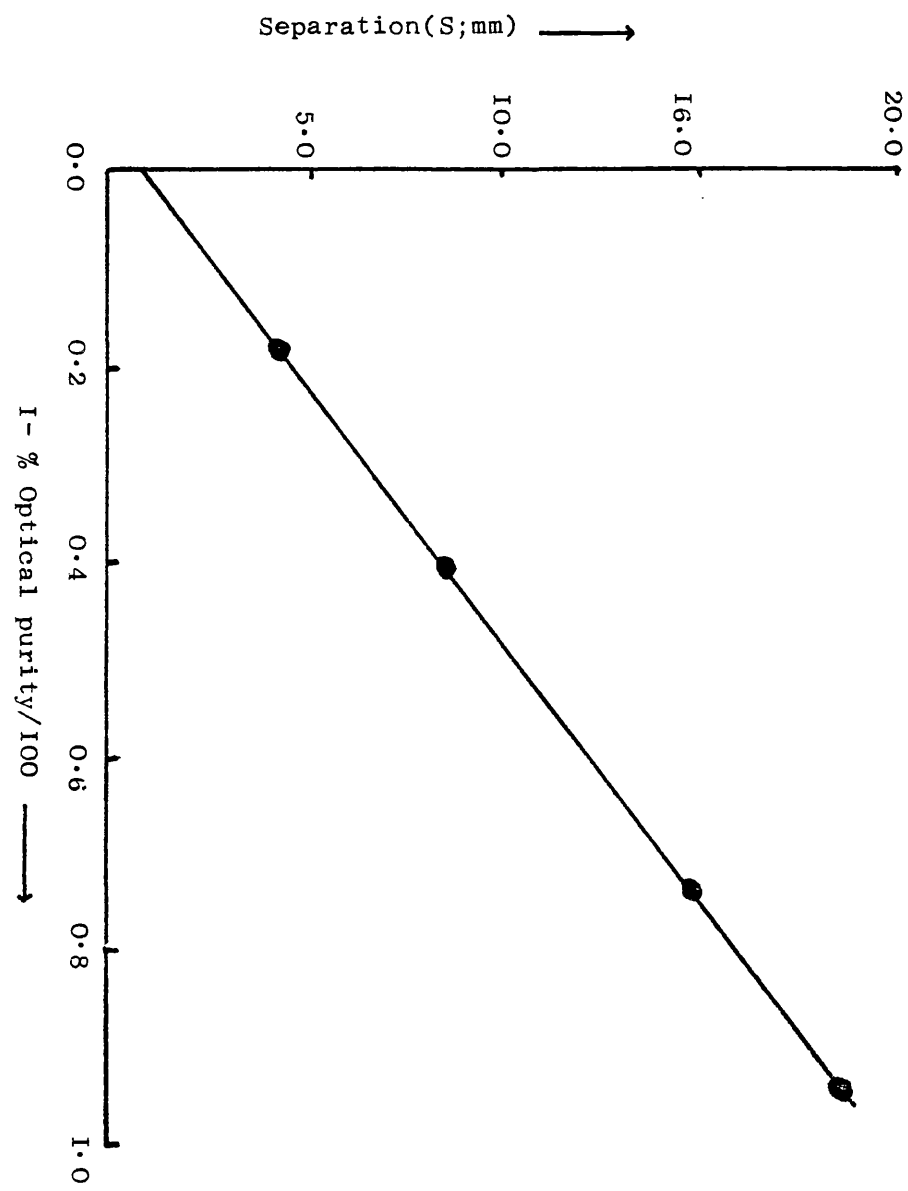


FIG. 2:37 Fenoprofen- Optical purity determination by the application of Base line technique.

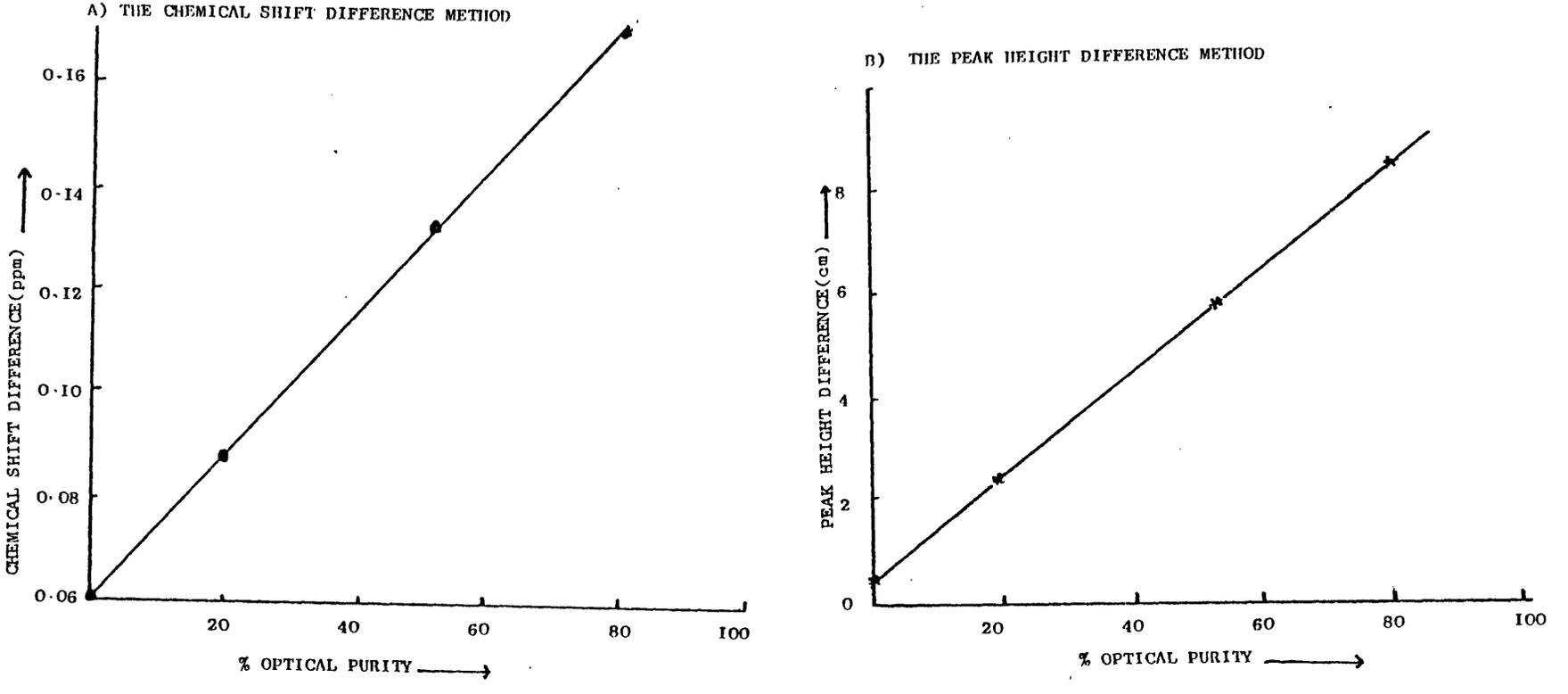
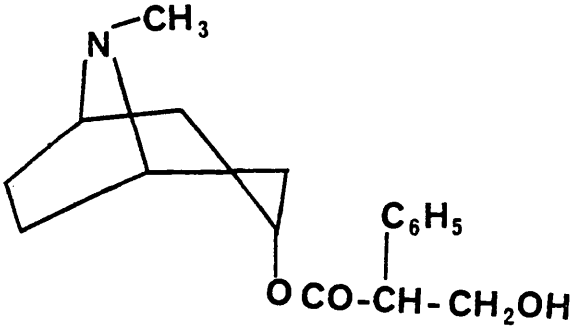


FIG.2:38 THE OPTICAL PURITY DETERMINATION OF FENOPROFEN BY THE CHEMICAL SHIFT DIFFERENCE & THE PEAK HEIGHT DIFFERENCE METHODS.

and in tables 2:22A - C. Of the three solvents chosen for the ΔLIS determination, CCl_4 was the most suitable (fig. 2:36). At higher reagent concentration, solubility in CCl_4 was a problem. However, at a molar ratio of 0.919 and a substrate concentration of 0.215 M the ΔLIS for the $COOCH_3$ signals was large enough for the optical purity determination (fig. 2:35, page 147).

All three techniques adopted for the optical purity determination proved suitable for the analysis. The results for the optical purity determination are given in table 2:23 (page 146), and in figures 2:37 (page 149) and 2:38 (page 150). The linearity of the graphs is an indication of the suitability of the techniques.

2.2.10 ATROPINE/HYOSCYAMINE



(2:21)

Atropine dl-hyoscyamine (2:21) and l-hyoscyamine (2:21) are alkaloids obtained from various solanaceous plants such as *Atropa belladonna* for atropine, and *Hyoscyamus muticus* or *Duboisia myoporoides* for hyoscyamine. They are also prepared synthetically[152]. They are used medicinally as parasympatholytic agents, particularly as antispasmodics[153].

The optical purity of atropine/hyoscyamine has been determined

Table 2:24A LIS for the CH_2O and $N-CH_3$ signals of atropine in $CDCl_3$ using $Eu(fod)_3$

[Atropine] = 0.174 M

MOLAR RATIO $Eu(fod)_3$ / Atropine	CH_2O δ	CH_2O LIS	$N-CH_3$ δ	$N-CH_3$ LIS
0.0	2.95	0.00	2.17	0.00
0.148	3.32	0.37	2.48	0.31
0.378	4.12	1.17	3.15	0.98
0.442	4.86	1.91	3.78	1.61
0.633	6.00	3.05	4.74	2.57

Table 2:24B ΔLIS for the $COOCH_3$ signals of tropic acid methyl ester in CCl_4 using $Eu(facam)_3$

* [Substrate] = 0.255 M

MOLAR RATIO	Chemical shift (δ-scale, ppm) l-isomer	d-isomer	ΔΔν (ppm)
0.0	3.64	3.64	0.00
0.246	4.70	4.70	0.00
0.440	5.50	5.50	0.00
0.565	5.93	5.90	0.03
0.728	6.37	6.29	0.08
0.876	6.70	6.58	0.12
1.010	7.07	6.89	0.18

* Substrate - tropic acid methyl ester.

Table 2:24C ΔLIS for the $COOCH_3$ signals of tropic acid methyl ester in C_6D_6 using $Eu(facam)_3$

[Substrate] = 0.295 M

MOLAR RATIO	Chemical shift (δ-scale, ppm)		ΔΔv (ppm)
	l-isomer	d-isomer	
0.0	3.22	3.22	0.00
0.186	3.81	3.81	0.00
0.429	4.58	4.54	0.04
0.522	4.84	4.80	0.04
0.641	5.12	5.08	0.04

Table 2:25 Optical purity determination of atropine/hyoscyamine using tropic acid methyl ester

% Optical purity (OP)	$1 - \frac{O.P.}{100}$	Separation (S; mm)	Peak height difference (l-d, mmm)	Chemical shift difference (δv; ppm)
0.0	1.00	10	-0.2	0.144
18.2	0.82	8.2	7.5	0.160
46.3	0.54	5.3	19.5	0.195
63.8	0.36	3.6	26.5	0.215
100.0	0.00	0.0	42.8	-

Solvent: CCl_4

Reagent: $Eu(facam)_3$

Substrate: tropic acid methyl ester

Molar ratio: Reagent / Substrate : 1.00

Analytical peaks: $COOCH_3$ at δ6.8 and δ7.8

Table 2:26 Relationship between concentration of tropic acid methyl ester (TAME) and separation (S)

Molar Ratio: $Eu(facam)_3$ / T A M E. = 1.00

Solvent: CCl_4

Analytical peak: $COOCH_3$ signal

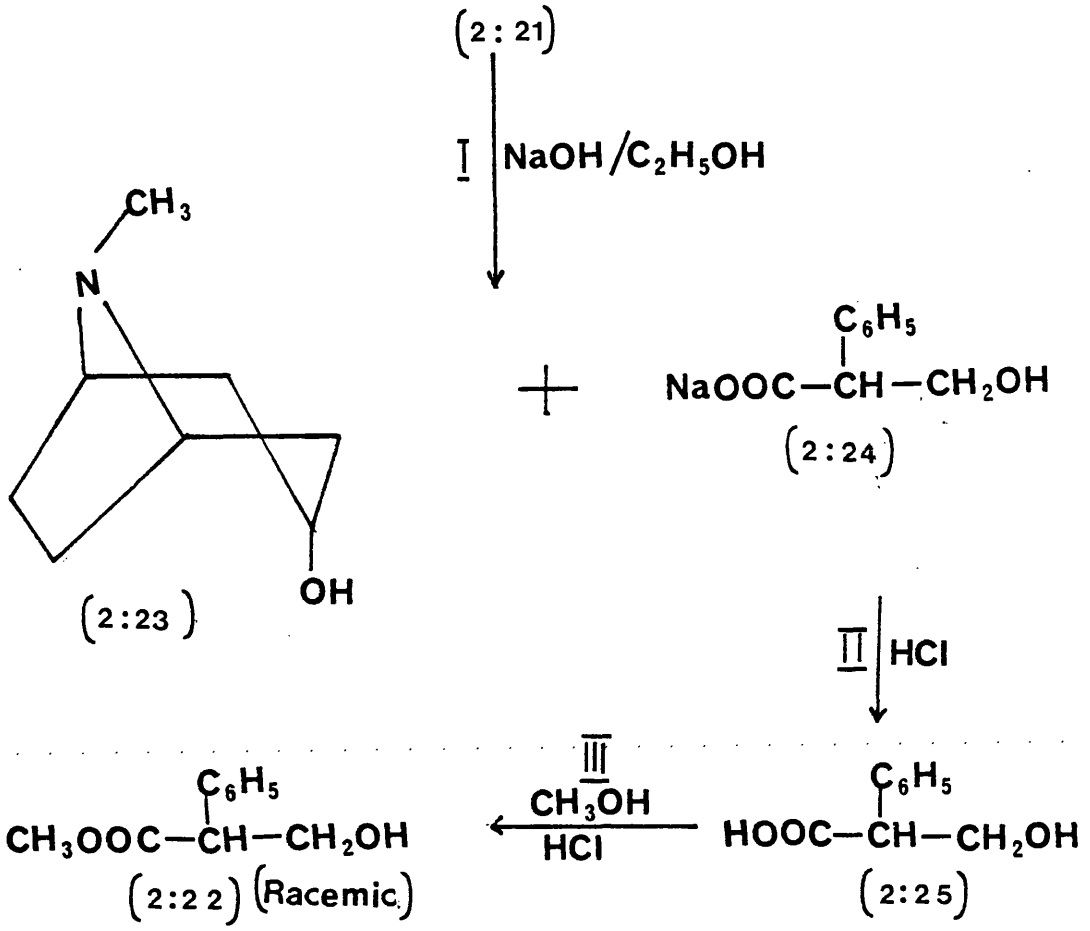
Concentration of tropic acid methyl ester (T A M E.)	Separation (S; mm)
0.201 M	6.0
0.240 M	7.8
0.282 M	10.0
0.296 M	10.6
0.301 M	11.0

Table 2:27 Relationship between 'broadness' of the $COOCH_3$ signal of tropic acid methyl ester and molar ratio using $Eu(fod)_3$ in CCl_4

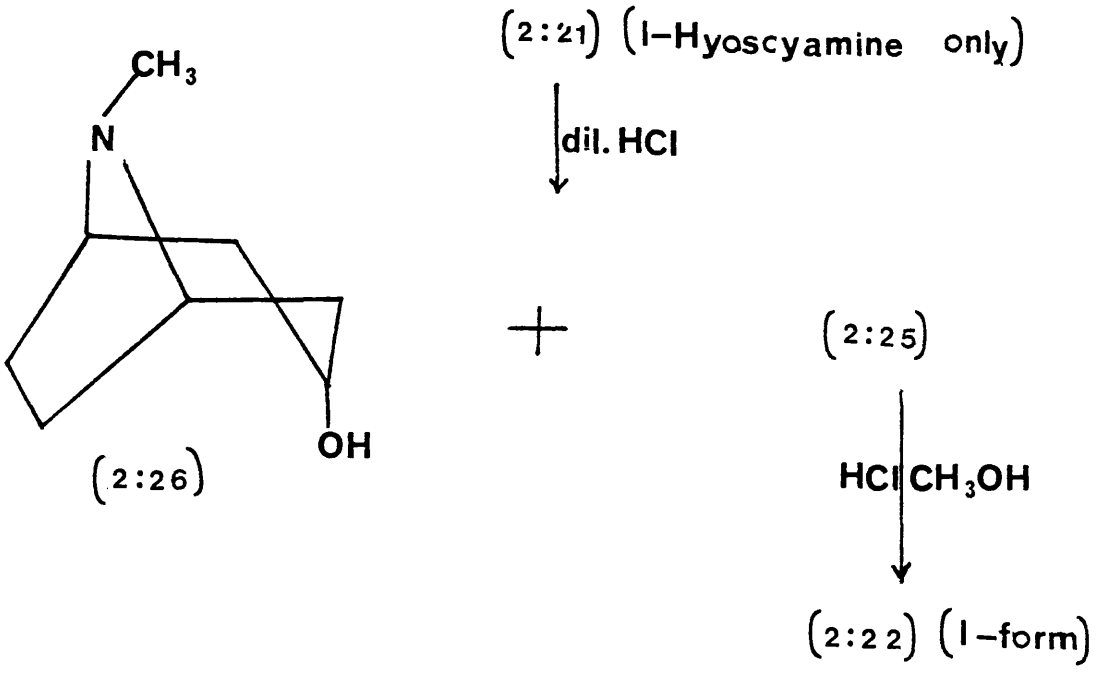
[TAME] = 0.237 M

MOLAR RATIO	Chemical shift δ (ppm)	LIS (ppm)	Width of OCH_3 signal at ½ height (mm)	Height of $COOCH_3$ signal (cm)
0.0	3.64	0.00	0.90	9.41
0.145	4.20	0.56	1.1	8.80
0.240	4.65	1.01	1.3	8.21
0.336	5.11	1.47	1.5	8.02
0.491	5.64	2.00	2.0	7.84
0.637	6.16	2.52	2.4	5.75

Scheme 2 : 5



Scheme 2 : 6



by the use of $Eu(facam)_3$ and tropic acid methyl ester (2:22) in CCl_4. The atropine/hyoscyamine itself could not be used for the analysis because there was no suitable resonance. (2:22) was synthesised from atropine/hyoscyamine (schemes 5 and 6, page 155). The $COOCH_3$ signal of (2:22) was employed for the analysis. Results for the LIS and ΔLIS are given in tables 2:24A - C (pages 152 & 153) and in figure 2:40 (page 158).

2.2.10.1 Results and Discussion

Synthesis of the l-tropic acid methyl ester (2:22) from the l-hyoscyamine presented some difficulties (unlike that from atropine). Hydrolysis of the hyoscyamine with NaOH and extraction with chloroform when hot results in complete racemisation. It is believed that the racemisation takes place at stage I of the synthetic route (scheme 2:5, page 155), and during extraction with chloroform[154]. To overcome this problem, acid hydrolysis is employed for hyoscyamine, using ether extraction. When chloroform is used as extracting solvent the solution must be allowed to cool completely before the extraction. It has been reported[154] that during the extraction of l-hyoscyamine, partial racemisation takes place. Complete racemisation takes place on treatment with alkali or heating in chloroform solution. Similar observations on the l-tropic acid has been made since 1919[155,156].

The problem of racemisation is critical and this approach of optical purity determination of atropine/hyoscyamine (through the formation of tropic acid methyl ester) is liable to false results if the right experimental conditions are not employed during synthesis.

FIG. 2 : 39 TROPIC ACID METHYL ESTER (0·2 M) IN CCl_4

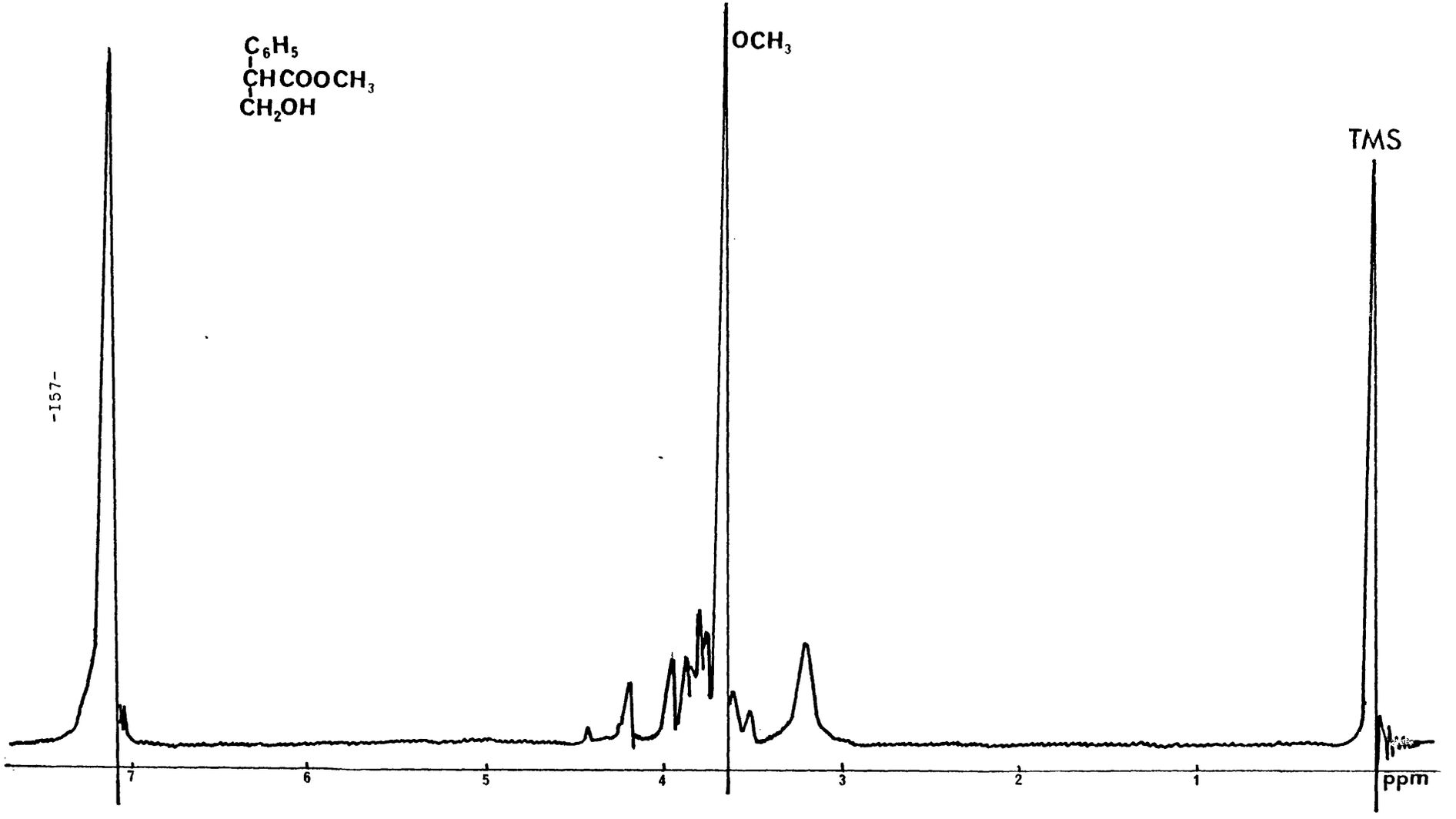


FIG. 2:40 Tropic acid methyl ester- OCH_3 signal

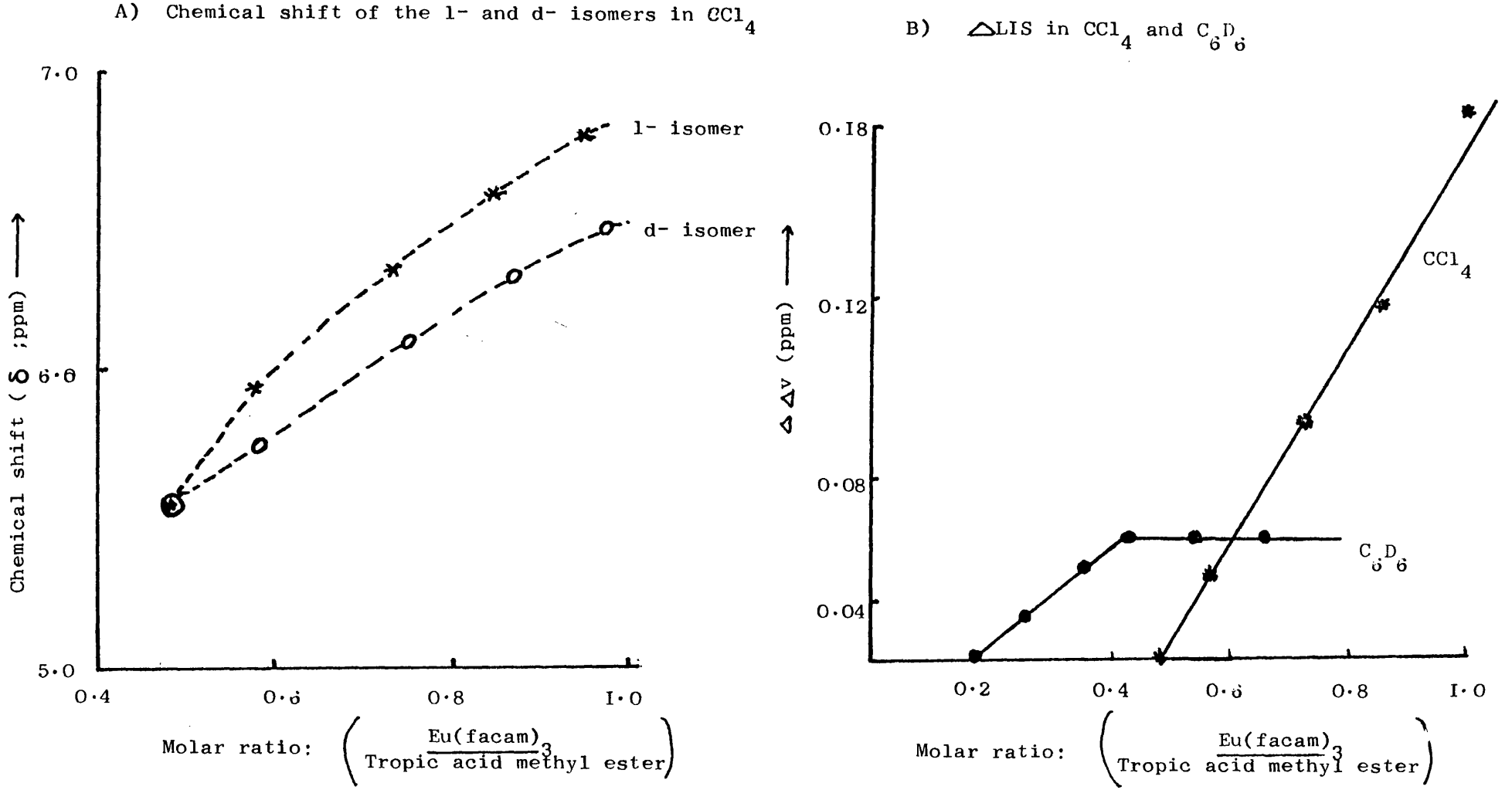
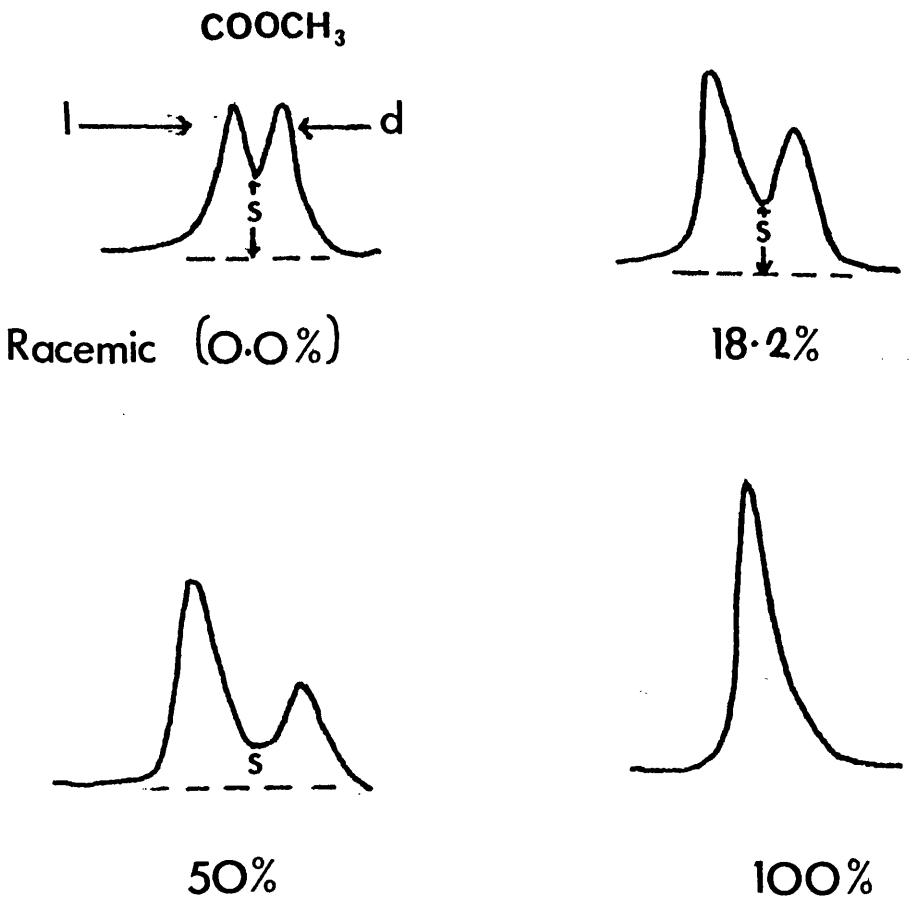


FIG. 2:41 The Base line technique for Optical purity determination of Atropine/Hyoscyamine



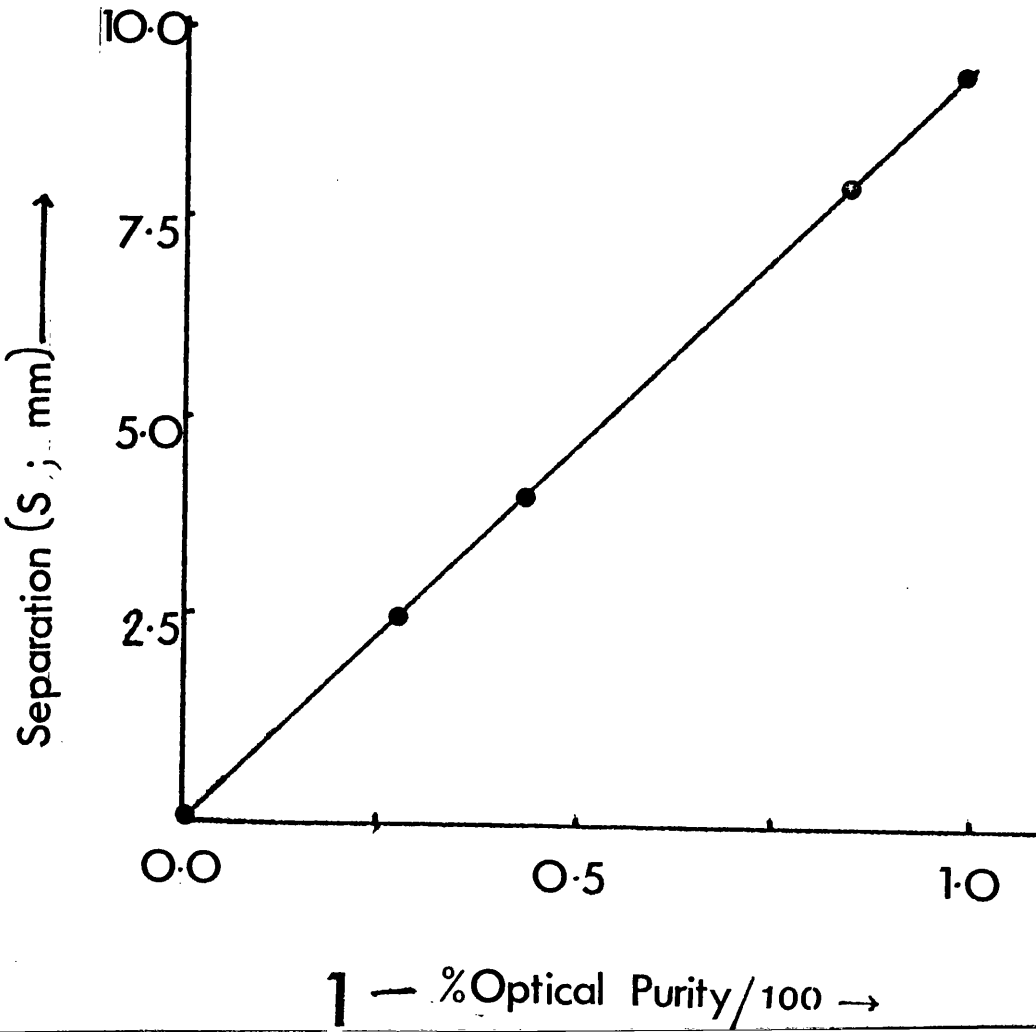
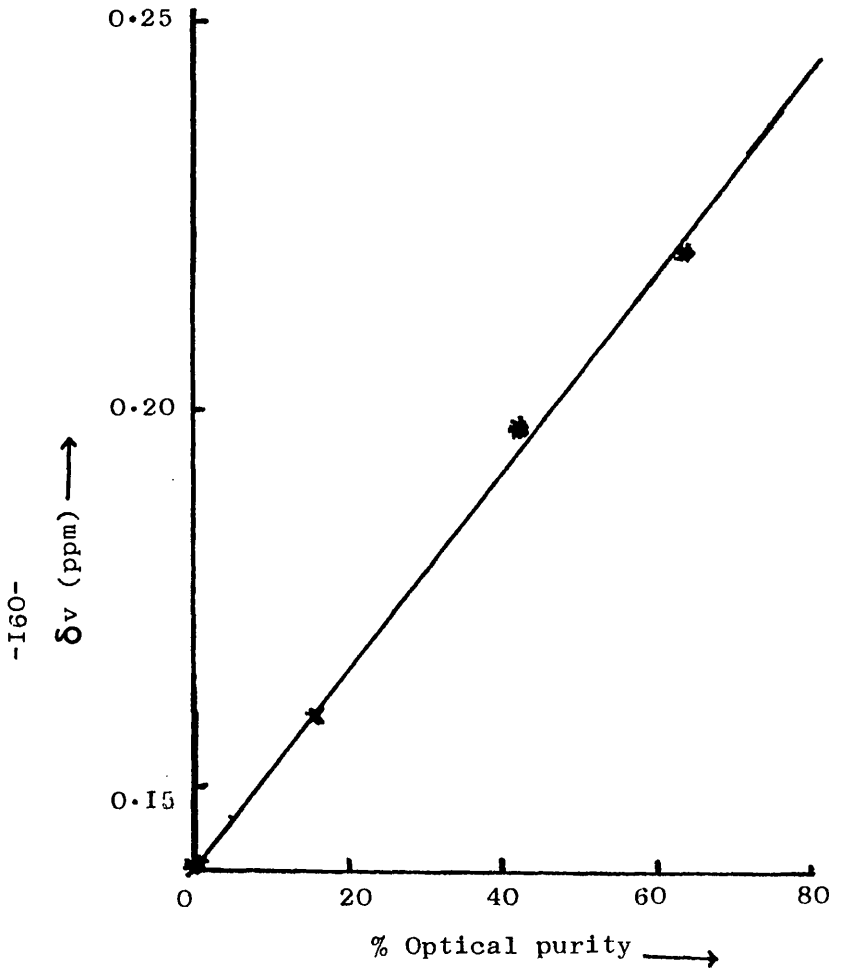
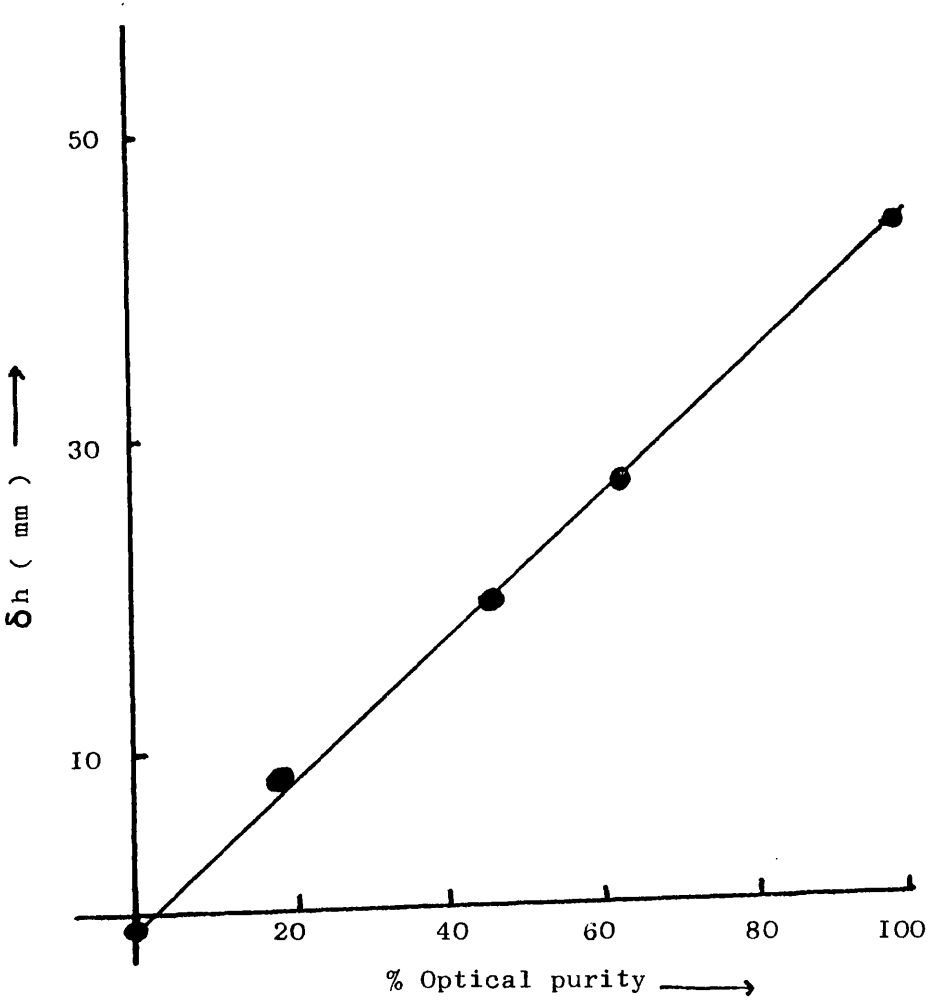


FIG. 2:42 Atropine/ hyoscyamine Optical Purity determination:

A) Chemical shift difference method



B) Peak height difference method

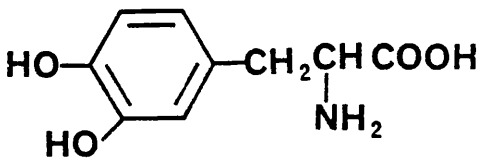


This clearly points out one of the dangers involved in the use of derivatives instead of the parent compound for optical purity determination.

Three solvents, $CDCl_3$, C_6D_6 and CCl_4, were considered for the analysis but only CCl_4 was found suitable. Due to the incomplete separation of the $COOCH_3$ peaks for the two isomers (l and d), integration could not be used. The usual three techniques used for the analysis gave linear relationships with the appropriate parameter. A correlation coefficient greater than 0.98 was obtained in each case. The results are shown in figure 2:41 (page 159) and 2:42 (page 160) and in table 2:25 (page 153).

The relationship between the substrate concentration and the separation (S) is given in table 2:26 (page 154). A discussion on this is covered in the general discussion, page 42 . Table 2:26 (page 154) shows the relationship between broadness of resonance peaks and the molar ratio. A discussion of this is also given in the general discussion, page 37 .

2.2.11 DOPA



(2:27)

Dopa (3,4-Dihydroxyphenylalanine)(2:27) is an amino acid found in seedlings, pods and beans of *Vicia faba* L. (broad beans), *Stizolobium deeringianum* L. (velvet beans), *Leguminosae*[157]. It is readily soluble in dilute acids and alkalis, and practically insoluble in absolute alcohol, ether, glacial acetic acid, petroleum ether and chloroform. Levodopa (L-Dopa) the naturally occurring amino acid is the immediate precursor of the synaptic neurotransmitter substance dopamine. Unlike dopamine, Levodopa readily enters the central nervous system and has been used in the treatment of conditions such as Parkinson's disease, which may result from a depletion of dopamine in the brain. Levodopa has also been used to control the neurological symptoms of chronic manganese poisoning, which resemble those of parkinsonism[158].

The enantiomeric ratio of Dopa has been determined using the $(TMS)_3$-Dopa methyl ester (2:30) and $Eu(facam)_3$ in CCl_4. Dopa itself could not be used because of insolubility in common organic solvents. The methyl ester (2:28) was synthesised (scheme 2:7) but could not be used directly, because of insolubility. The $(TMS)_4$ derivative of dopa itself was soluble in the common organic solvents but there was no suitable resonance peak for the analysis (scheme 2:7). In view of this, the $(TMS)_3$-Dopa methyl ester (scheme 2:7) was prepared and used for the analysis, employing the $COOCH_3$ signal. The results for LIS and the optical purity determinations are shown in tables 2:28A - C and 2:29 (page 164) and in figures 2:44 (page 167) and 2:45 (page 163).

Scheme 2:7

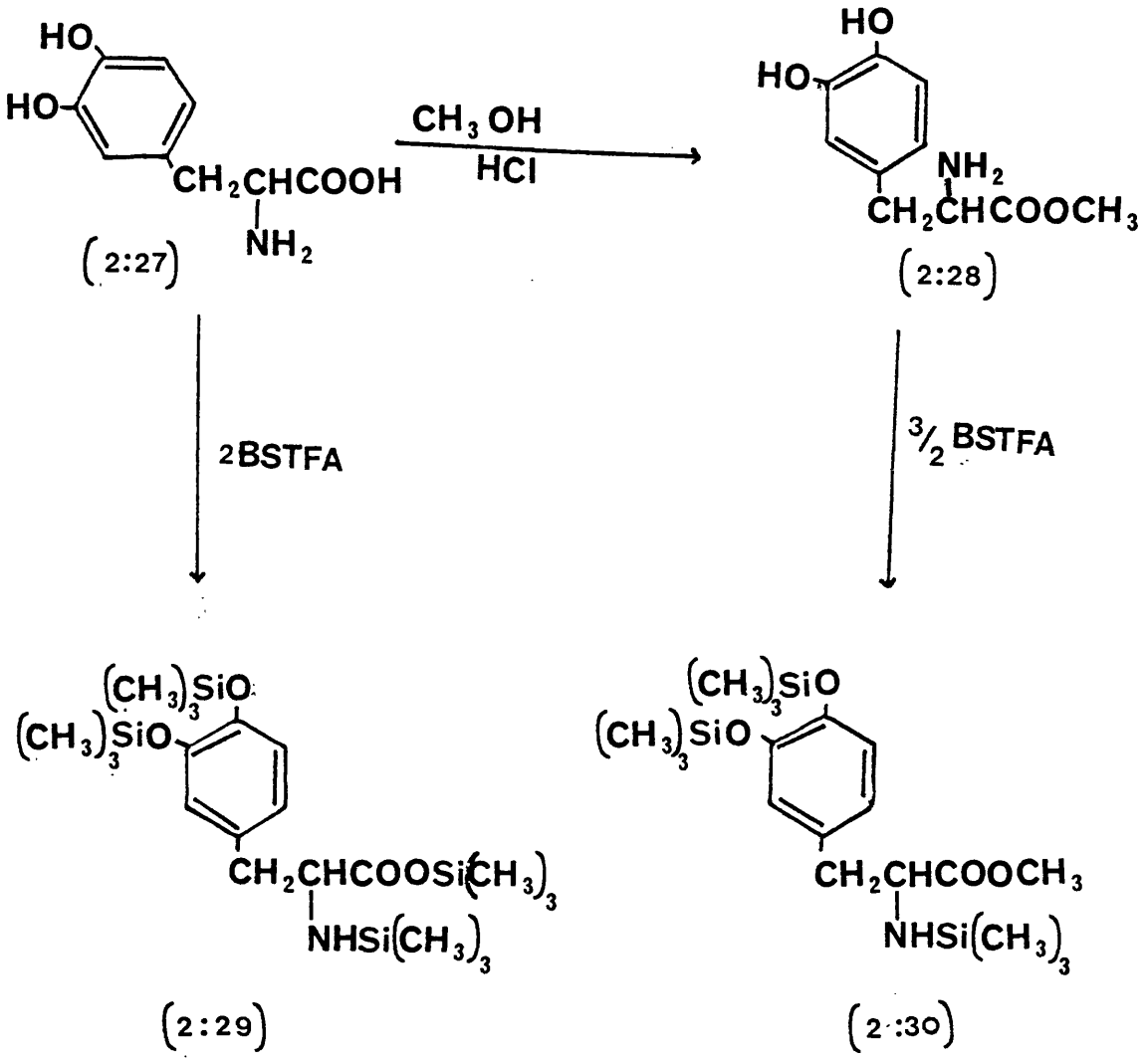

HO
HO
CH2CHCOOH
NH2
(2:27)
CH3OH
HCl
HO
HO
NH2
CH2CHCOOCH3
(2:28)
2BSTFA
3/2 BSTFA
(CH3)3SiO
(CH3)3SiO
CH2CHCOOSi(CH3)3
NHSi(CH3)3
(2:29)
(CH3)3SiO
(CH3)3SiO
CH2CHCOOCH3
NHSi(CH3)3
(2:30)

Table 2:28A LIS of the $COOCH_3$ signal of $(TMS)_3$-Dopa methyl ester in $CDCl_3$ using $Eu(fod)_3$

*MOLAR RATIO $\frac{Eu(fod)_3}{Substrate}$	Chemical shift (δ; ppm)	LIS (Δv; ppm)
0.0	3.61	0.00
0.082	4.01	0.40
0.169	4.35	0.74
0.246	4.71	1.10
0.322	5.23	1.62

Table 2:28B $\Delta\Delta v$ of the $COOCH_3$ signal of $(TMS)_3$-Dopa methyl ester using $Eu(facam)_3$ in $CDCl_3$

*MOLAR RATIO $\frac{Eu(facam)_3}{Substrate}$	d-Dopa		l-Dopa		
	δ (ppm)	Δv (ppm)	δ (ppm)	Δv (ppm)	$\Delta\Delta v$ (ppm)
0.0	3.61	0.0	3.61	0.0	0.00
0.148	3.65	0.04	3.65	0.04	0.00
0.263	4.65	1.04	4.65	1.04	0.00
0.423	5.32	1.71	5.32	1.71	0.00
0.573	6.12	2.51	6.05	2.44	0.07
0.691	6.39	2.78	6.23	2.62	0.16

*The molar ratio calculation was based on the weight of Dopa- methyl ester (60 mg)

Table 2:28C $\Delta\Delta v$ for the $COOCH_3$ signal of $(TMS)_3$-Dopa methyl ester using $Eu(facam)_3$ in CCl_4

MOLAR RATIO $Eu(facam)_3$ / Substrate	d-Dopa		l-Dopa		
	δ	Δv	δ	Δv	$\Delta\Delta v$
0.0	3.58	0.0	3.58	0.0	0.00
0.176	4.11	0.53	4.11	0.53	0.00
0.345	4.59	1.01	4.59	1.01	0.00
0.492	5.21	1.63	5.21	1.63	0.00
0.609	2.74	2.16	5.74	2.16	0.00
0.842	6.09	2.51	5.89	2.31	0.20

Table 2:29 Optical purity determination of Dopa using TMS-Dopa methyl ester

% Optical purity	Base line technique		δh method	δv method
	l - OP/100	Separation (S; mm)	(l-d) (mm)	δv(ppm)
0.0	1.00	6.8	0.1	0.19
20.0	0.80	4.8	5.2	0.23
51.8	0.48	3.0	12.3	0.28
80.0	0.20	1.3	18.8	0.34
100.0	0.0	0.0	23.4	-

Solvent: CCl_4

Reagent: $Eu(facam)_3$

*Molar ratio: Reagent / Substrate = 0.842

Substrate: TMS-Dopa methyl ester

Analytical peaks: $COOCH_3$ at δ5.80 - 6.1 ppm

*The calculation of the molar ratio was based on the weight of the Dopa methyl ester (60 mg)

2.2.11.1 Discussion

The two solvents, $CDCl_3$ and CCl_4, employed for the analysis proved equally suitable (*cf.* tables 2:28B and 2:28C, pages 164 and 165 respectively). Thus, CCl_4 was used for the optical purity determination. Linear graphs were obtained for all the three techniques employed for the optical purity determination (figures 2:44 and 2:45).

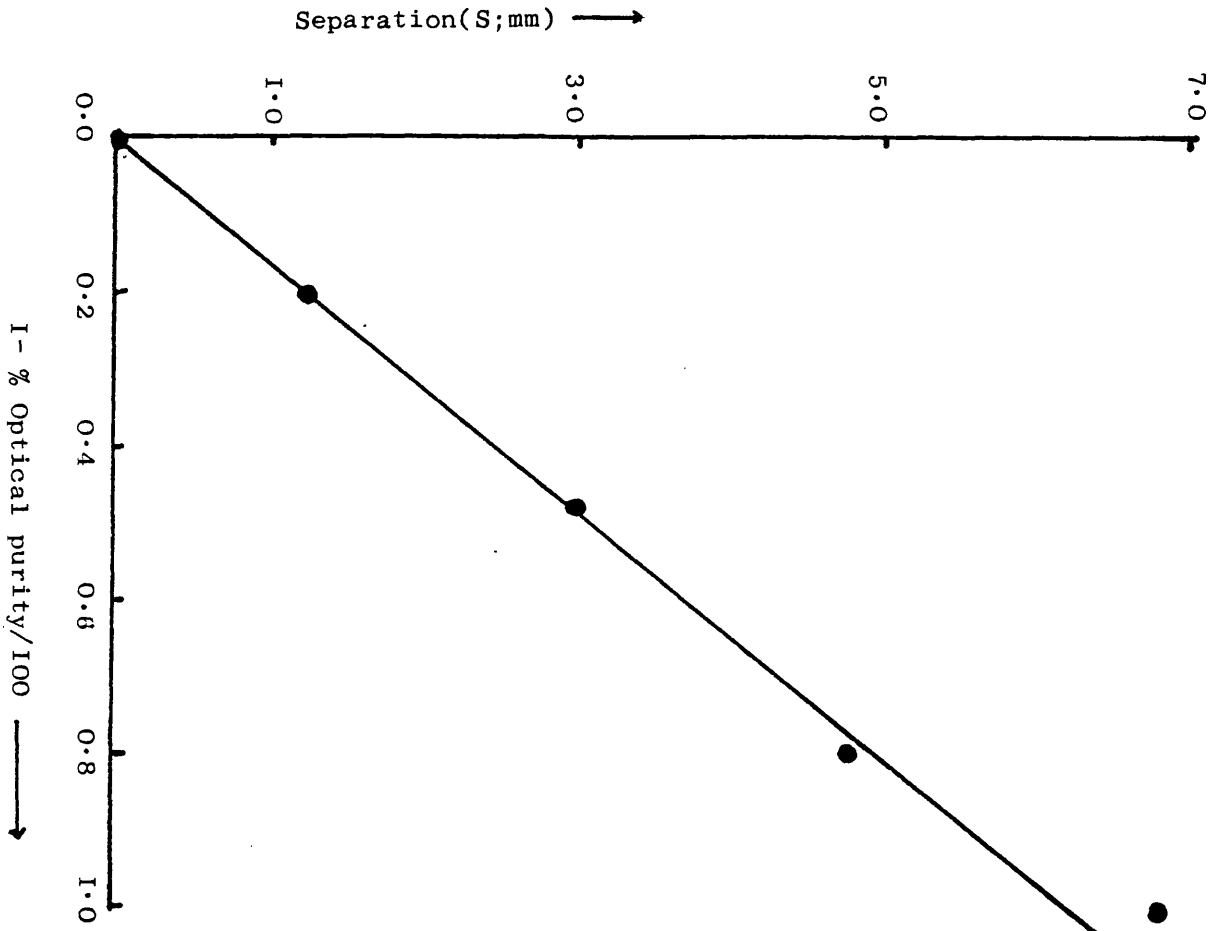


FIG. 2:44 Optical purity determination of Dopa by the use of Base line technique

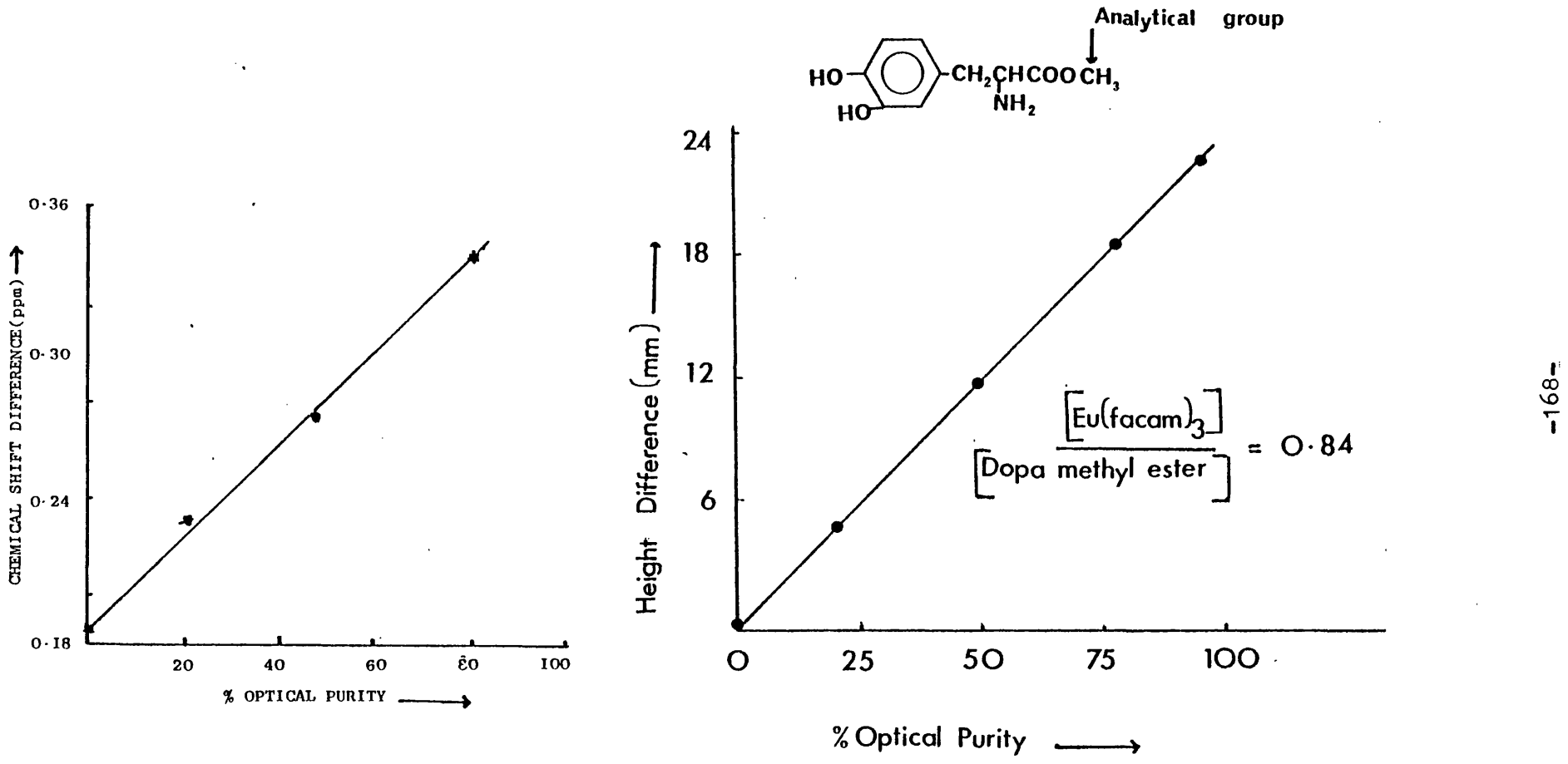
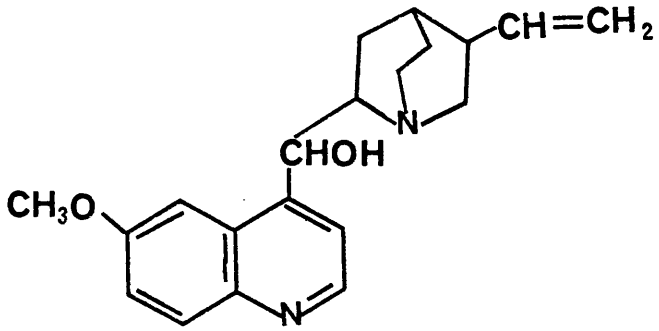


FIG. 2:45 THE OPTICAL PURITY DETERMINATION OF DOPA BY THE CHEMICAL SHIFT DIFFERENCE & THE PEAK HEIGHT DIFFERENCE METHODS.

2.3 DIASTEREOISOMERS

2.3.1 QUININE AND QUINIDINE



(2:31)

Quinine [α-(6-methoxyquinolin-4-yl)-α-(5-vinyl-quinuclidin-2-yl) methanol] (2:31), ($C_{20}H_{24}N_2O_2 \cdot 3H_2O$) is the chief alkaloid of various species of *Cinchona (Rubiaceae)*. It is a stereoisomer of quinidine and normally exists as the trihydrate. It is a highly active blood Schizonticide and suppresses the asexual cycle of development of the malaria parasite in the erythrocytes. It has some analgesic and antipyretic properties and can also be used to induce labour and reduce night cramps[158].

Quinidine (2:31) $C_{20}N_{24}N_2O_2 \cdot 2H_2O$ is a dextrorotatory stereoisomer of quinine. It is also obtained from the back of *Cinchona* species. It normally exists as the dihydrate. It is a cardiac depressant, reducing the cardiac activity by directly depressing the excitability of the myocardium and prolonging the refractory period of cardiac muscle, thereby reducing the rate at which successive contractions take place. It also has anticholinergic actions[158].

The diastereomeric ratio of quinine and quinidine (of prepared mixtures) has been determined without a shift reagent, and also by the

use of $Pr(fod)_3$ a non-chiral lanthanide shift reagent.

2.3.1.1 Results and Discussion

The chemical shifts of quinine resonance signals in $CDCl_3$ are slightly different from those of quinidine. The chemical shift difference for a particular group varies proportionally with a change in the diastereomeric ratio, without the use of a shift reagent (figures 2:46, page 176 and 2:47A, page 177). It was therefore possible to determine the diastereomeric ratio without the use of a shift reagent. However, the chemical shift difference (δv) was improved by the use of $Pr(fod)_3$.

Three techniques - the chemical shift difference, the base line and the peak height difference methods - were employed for the diasteromeric ratio determination.

(i) The chemical shift difference method:

At low concentrations, quinine and quinidine have slightly different chemical shifts which are just noticeable if equal amounts of both quinine and quinidine are present (figure 2:46, page 176). As the ratio (quinidine/quinine) increases the chemical shift difference also increases linearly. The reverse, quinine/quinidine, *vrs* δv is shown in figure 2:47A, page 177 . Thus, the diastereomeric ratio can be determined accurately by the use of the chemical shift difference. The results for the experiments are given in tables 2:30A - C, page 172).

It was found that the chemical shifts of both quinine and quinidine decreased with increase in concentration (fig. 2:47B, page 177).

However, the chemical shift of quinidine is more concentration dependent than those of quinine. Thus, when the amount of quinine was greater than that of quinidine in the mixture the chemical shift difference was very small. For the diastereomeric ratio determination in the absence of a shift reagent, the amount of quinidine must be greater than that of quinine in each case. In the presence of a shift reagent, the relative amounts are less important since the chemical shift difference is almost doubled by addition of $Pr(fod)_3$.

(ii) The base line technique:

An interesting result was obtained for the base line technique. The base line technique, which normally gives a linear plot, gave a parabola with or without a shift reagent. The results are shown in figure 2:48 (page 178). However, when the log S was plotted against the ratio quinine/quinidine, a straight line was obtained (figure 2:48B).

(iii) Peak height difference method:

Figure 2:49 shows the results for the peak height difference method. A linear plot for the peak height difference versus the molar ratio was obtained.

2.3.2 EPHEDRINE AND PSEUDOEPHEDRINE MIXTURE

A mixture of ephedrine and *pseudo* ephedrine could be determined readily by integration of the $-\overset{\text{O}}{\overset{|}{C}}H$ peaks. The OC*H* signal for ephedrine is separated from that of the *pseudo* ephedrine by about 0.5 ppm. Figure 2:50 (page 180) shows the nmr spectrum of a mixture of ephedrine and *pseudo* ephedrine. Though the other groups (e.g. NCH_3 and the $C\text{-}CH_3$ in ephedrine and *pseudo* ephedrine) have slightly different

Table 2:30A Determination of the diastereomeric ratio of quinine/quinidine in the absence of a shift reagent

Solvent: $CDCl_3$

Analytical peak: OCH_3

Quinine (Wt. in gm.)	Quinidine (Wt. in gm.)	Quinine / Quinidine ratio	Chemical shift difference ($\delta\nu$; ppm)	Peak height difference (Q'dine-Q'nine; cm)	Base line technique: Separation (S; mm)	Base line technique: log S
0.0327	0.0312	1.048*	-	-	-	-
0.0318	0.0310	1.026*	-	-	-	-
0.0292	0.0330	0.885	0.017	-0.80	42.2	1.6253
0.0276	0.0349	0.791	0.022	-0.22	31.3	1.4955
0.0260	0.0367	0.708	0.033	1.41	23.8	1.3766
0.0205	0.0419	0.489	0.049	2.58	13.7	1.1367
0.0179	0.0449	0.399	0.054	3.57	12.4	1.0934
0.0139	0.0515	0.270	0.067	5.05	7.1	0.8513
0.0068	0.0543	0.125	0.082	7.18	4.8	0.6812
0.0053	0.0573	0.093	0.089	7.37	3.7	0.5682

*Almost a single peak was obtained

Table 2:30B Determination of the diastereomeric ratio of quinine/quinidine in the presence of $Pr(fod)_3$

Solvent: $CDCl_3$ (0.7 ml)

Wt. of $Pr(fod)_3$: 32 mg

Quinine (wt. in gm)	Quinidine (wt. in gm)	Quinine / Quinidine ratio	Chemical shift difference (δv; ppm)	Peak height difference (Q'dine-Q'nine) (δh (cm)	Base line technique: Separation (S; mm)	Base line technique: log S
0.0327	0.0312	1.048	0.033	-0.59	38.9	1.5899
0.0276	0.0349	0.791	0.048	0.52	21.0	1.3225
0.0205	0.0419	0.489	0.078	3.00	8.8	0.9445
0.0139	0.0515	0.270	0.107	4.52	4.4	0.6455
0.0053	0.0573	0.092	0.134	7.85	1.6	0.2041

Table 2:30C Effect of concentration on the chemical shift of quinine and quinidine using the OCH_3 signal

Solvent: $CDCl_3$

QUININE		QUINIDINE	
Wt./ml.	Chemical shift (δ; ppm)	Wt./ml.	Chemical shift (δ; ppm)
48.3	3.853	47.2	3.80
104.1	3.847	104.1	3.79
150.0	3.846	150.0	3.78
200.4	3.844	200.1	3.77

chemical shifts they are insufficiently large for accurate integration. The base line technique and the chemical shift difference method have been used successfully to determine the ratio of ephedrine to *pseudo* ephedrine in a mixture of the two by using the N-CH_3 or the C-CH_3 signals at a scale expansion. Using the N-CH_3 signals, the region δ2.0 - 2.1 ppm was expanded on 100 Hz scale. For the C-CH_3, the expansion was at δ0.65 - 0.8 ppm.

Both the base line technique and the chemical shift difference gave a linear relationship when separation (S) or the chemical shift difference (δv) was plotted against the ratio of ephedrine to *pseudo* ephedrine. The results are given in table 2:31 and in figures 2:51 and 2:52 (pages 181 and 182).

Table 2:31 Determination of the diastereomeric ratio of ephedrine/ *pseudo* ephedrine

MOLAR RATIO ψ -Ephedrine / Ephedrine	N-CH_3 signal Separation (S; cm)	C-CH_3 signal Separation (S; cm)	C-CH_3 signal chemical shift difference (δv; Hz)
0.3916	1.37	0.60	1.82
0.4763	2.03	0.72	1.70
0.8341	5.10	1.50	1.00
1.0230	6.95	1.76	0.78

FIG. 2:46 OCH_3 signals at different Quinine/Quinidine ratios:

a) in the presence of $Pr(fod)_3$ (32mg.)

b) without shift reagent.

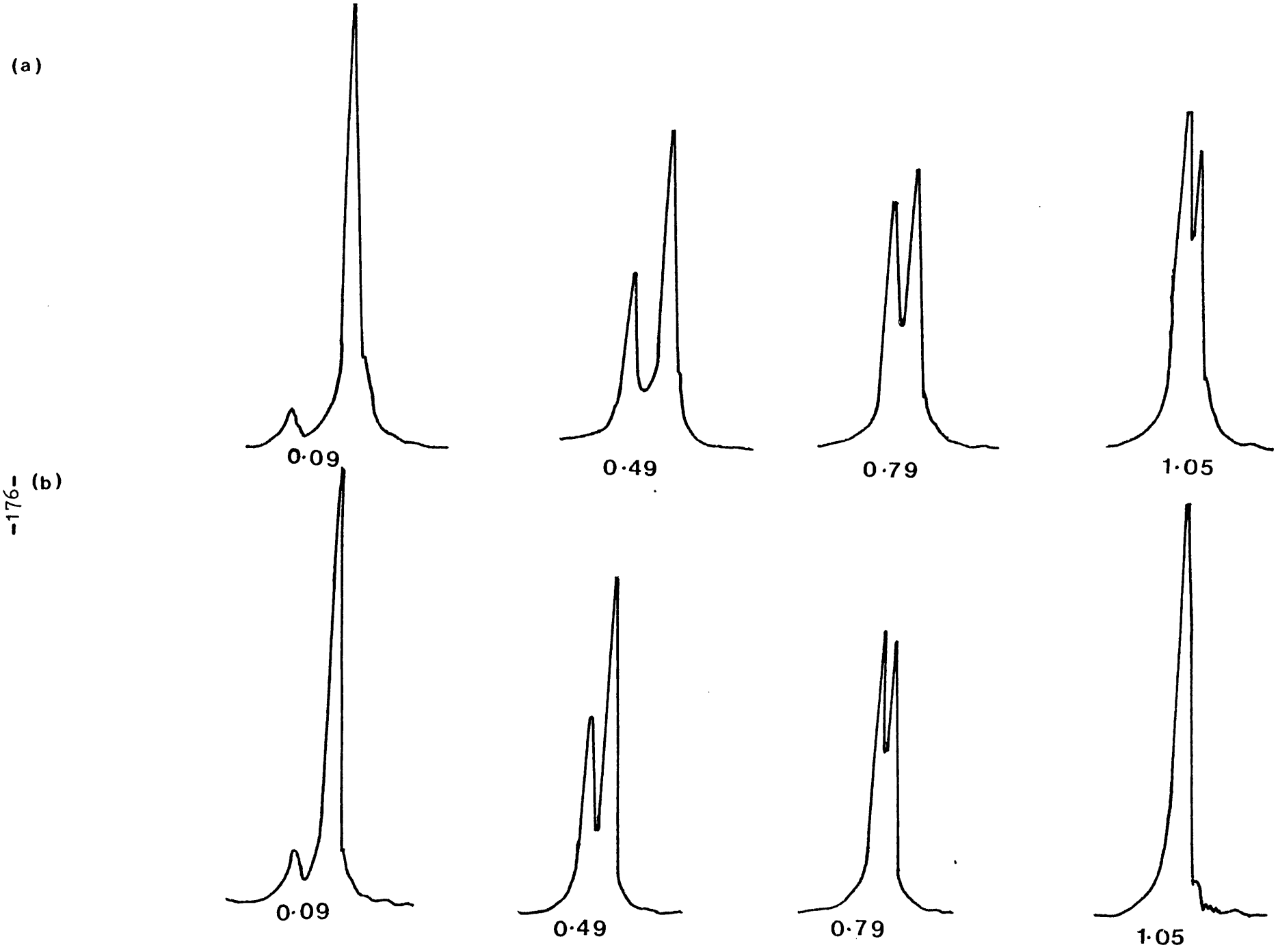
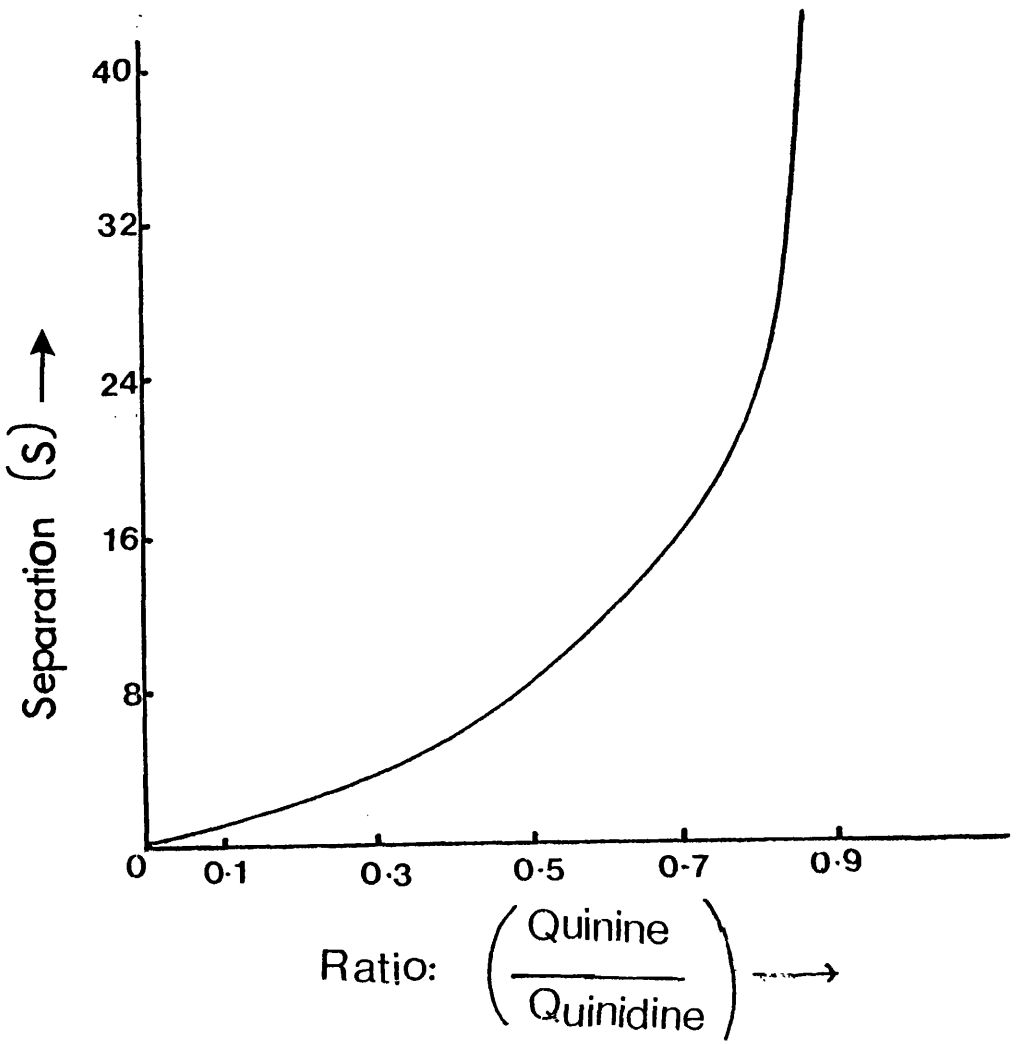
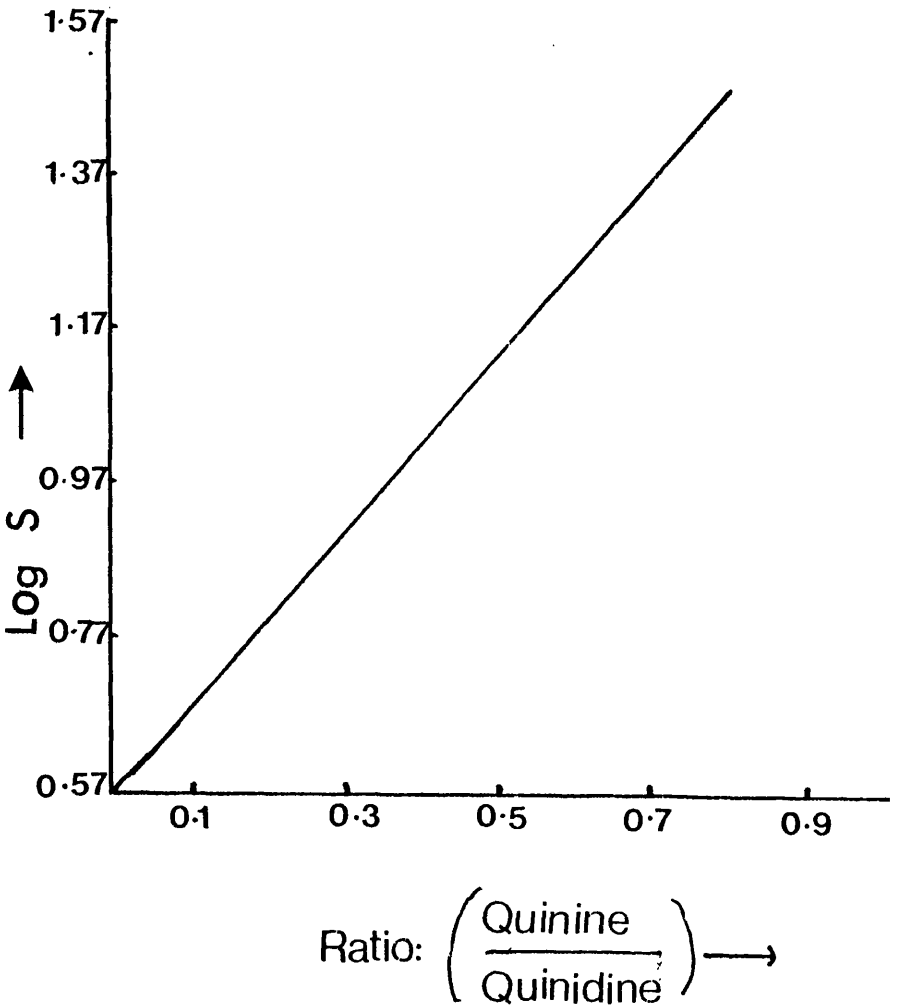

(a)
0·09
0·49
0·79
1·05
(b)
0·09
0·49
0·79
1·05

FIG. 2:48 The Base line technique for Diastereomeric ratio determination of Q'nine/Q'nidine





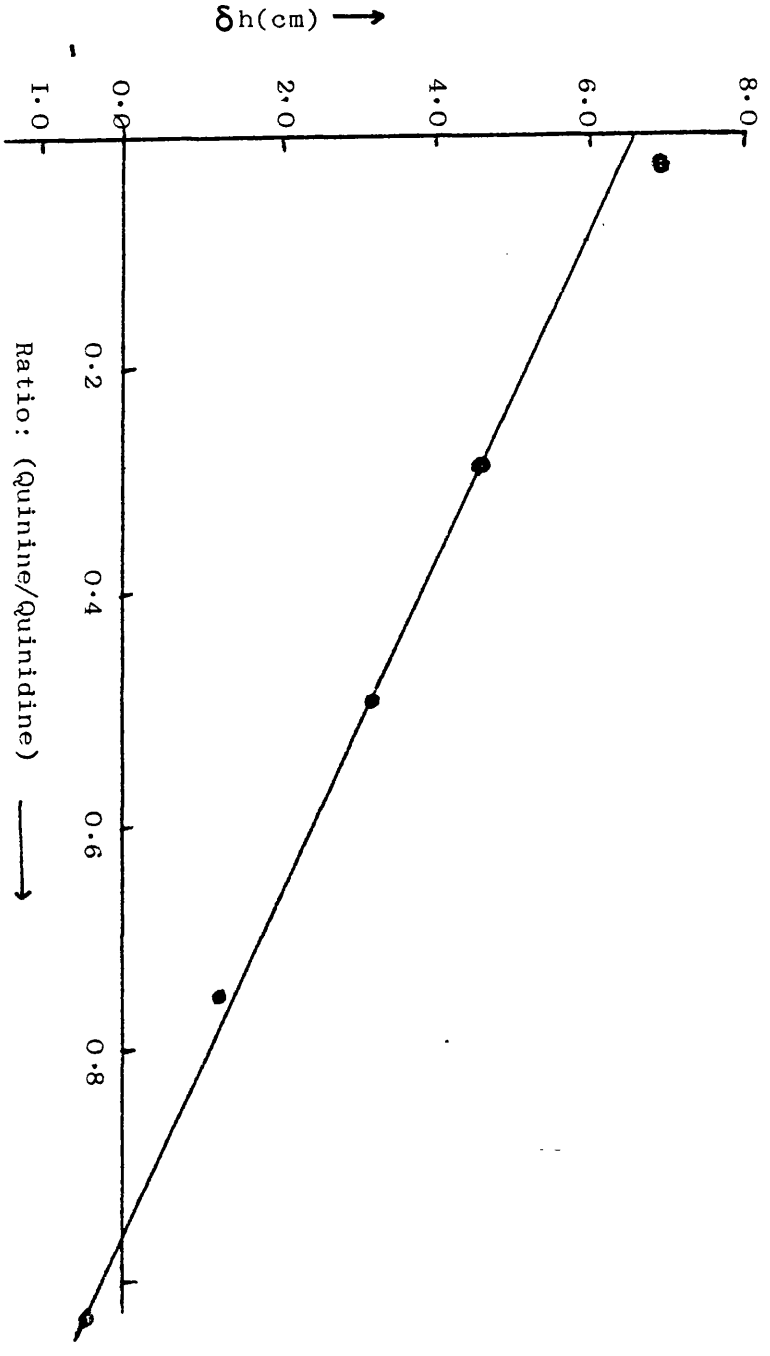


FIG. 2:49 Diastereomeric ratio determination of Quinine/Quinidine by peak height difference method.

FIG. 2:50 Ephedrine/Pseudo ephedrine (2:1) in C_6D_6

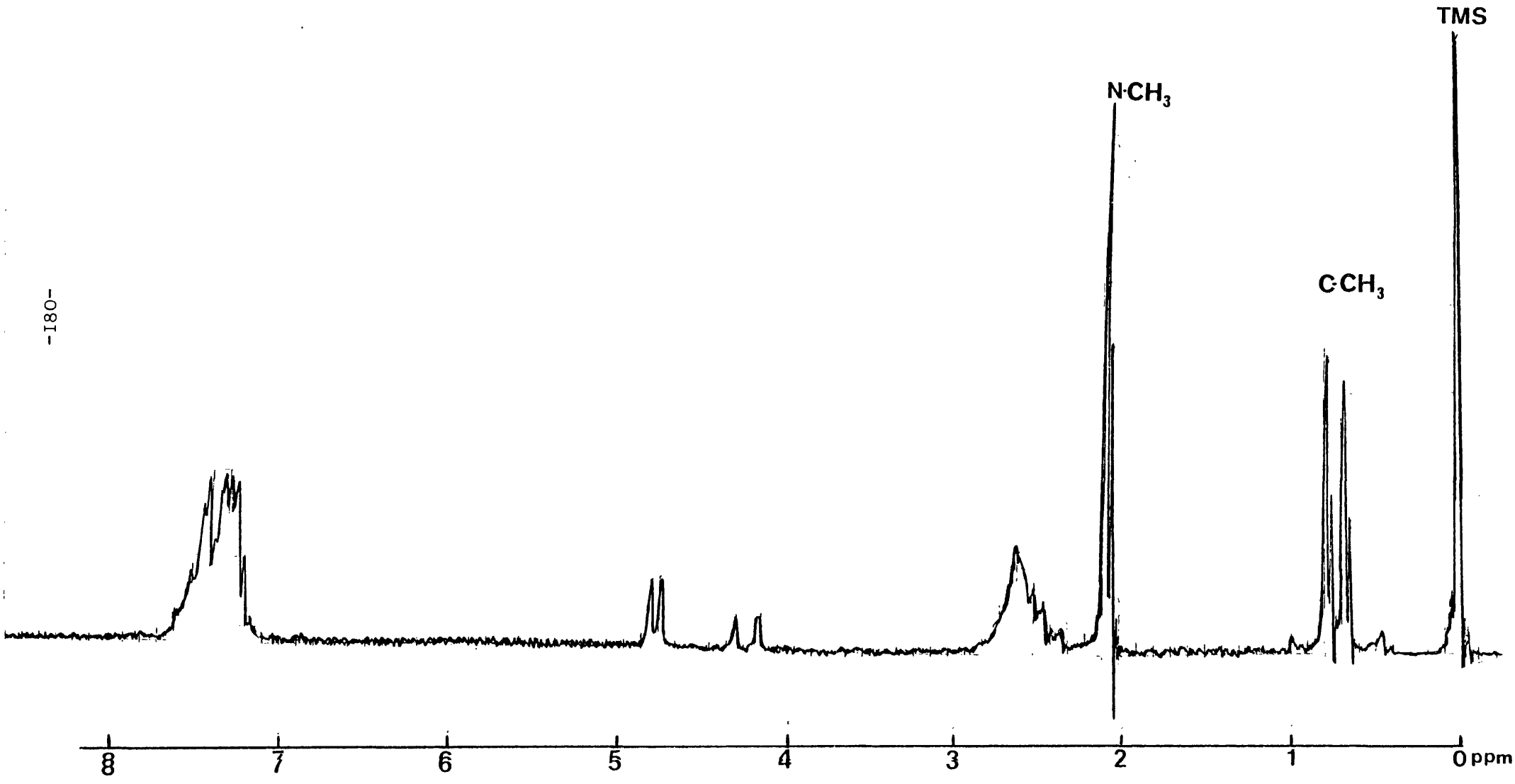


FIG. 2:5I Diastereomeric ratio determination of Ephedrine/Pseudo ephedrine mixture by the application of Base line technique.

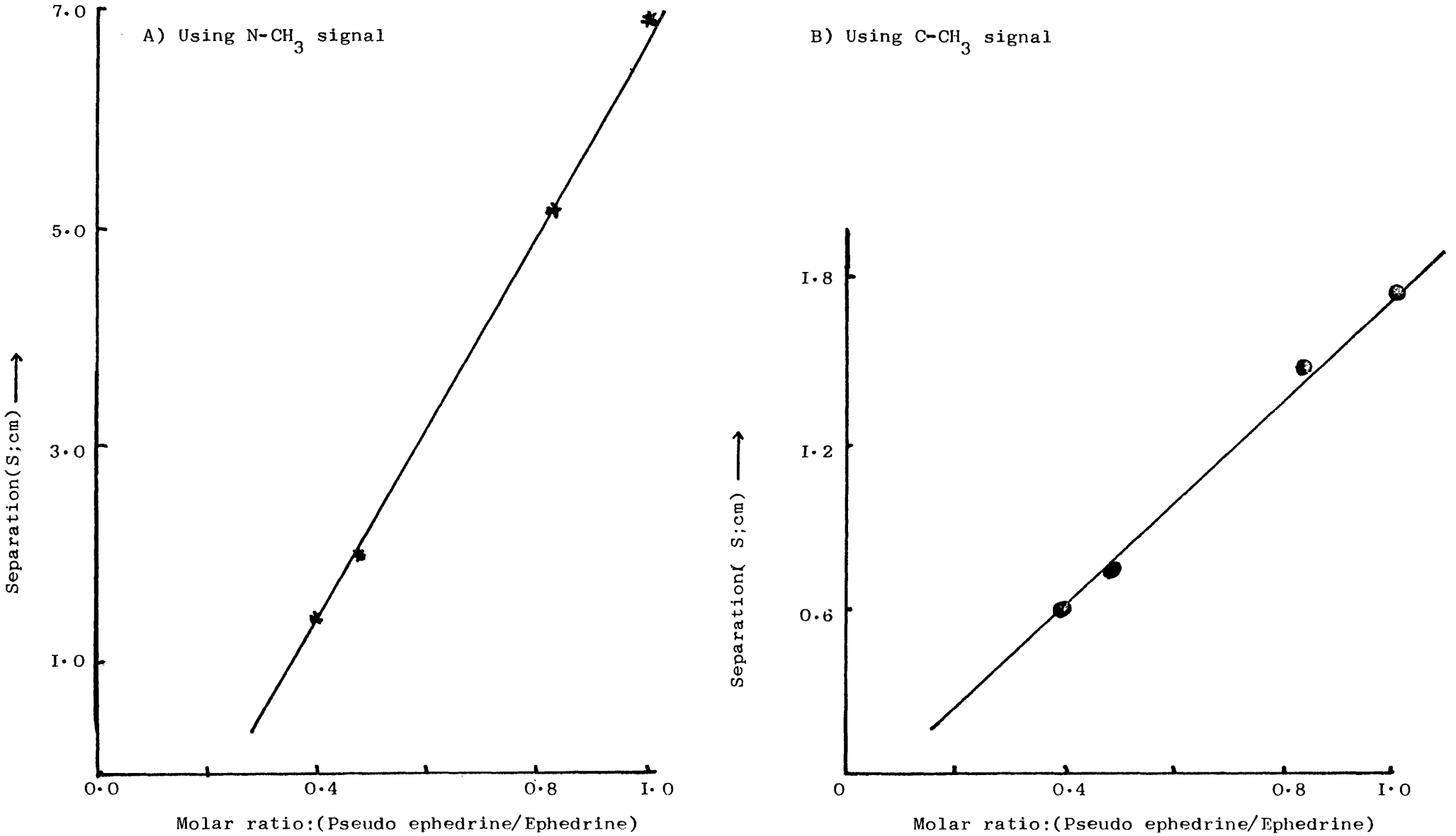
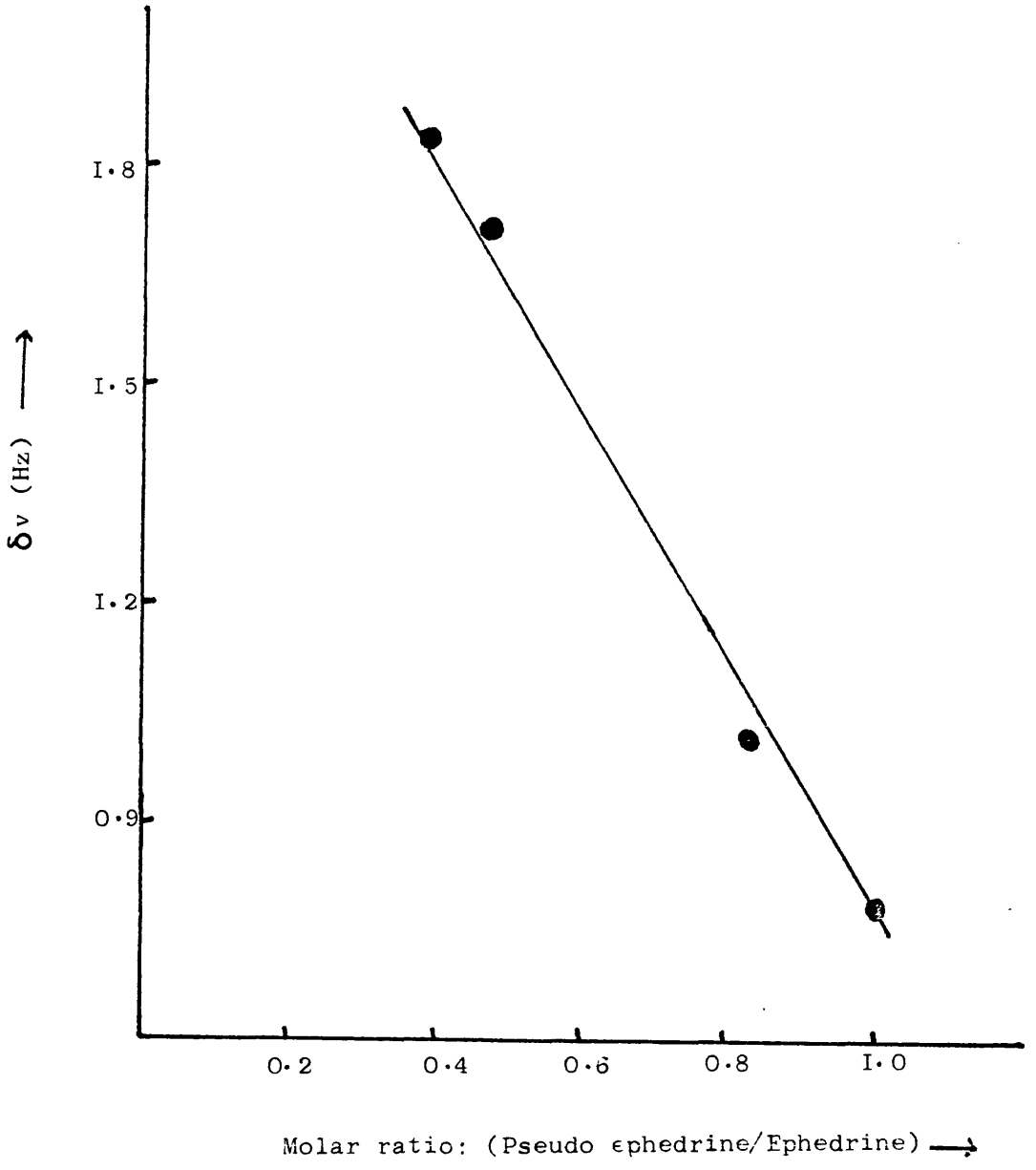
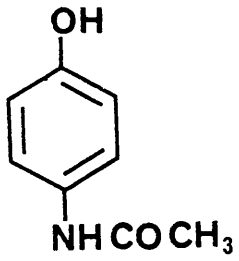


FIG: 2:52 Diastereomeric ratio determination of Ephedrine/Pseudo ephedrine mixture by the application of chemical shift difference method using the $N\text{-}CH_3$ signal.

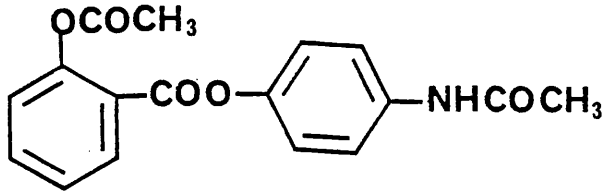


2.4 THE APPLICATION OF THE BASE LINE TECHNIQUE TO DISSIMILAR MOLECULES

2.4.1 BENORYLATE/PARACETAMOL MIXTURE



(2:32)

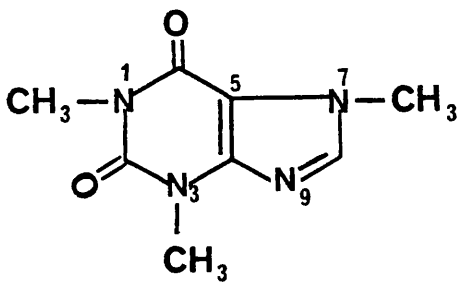


(2:33)

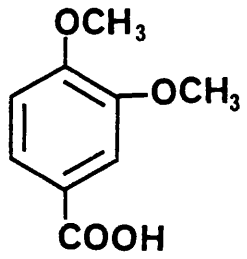
The N-COCH_3 signals for paracetamol (2:32) and benorylate (2:33) resonate close together δ2.07 and δ2.10 ppm respectively in $CDCl_3$/ DMSO(d_6) (2:1) and therefore form suitable analytical peaks for application of the base line technique to mixtures of two dissimilar molecules. A scale expansion at 100 Hz was used for the analysis. A linear relationship was obtained when the separation (S) was plotted against the molar ratio $\left(\frac{\text{Paracetamol}}{\text{Benorylate}}\right)$. The results are given in table 2:32 (page 185) and in figure 2:53 (page 187). The results show that when the total weight of the mixture is known, it is possible to obtain the exact weight of each component in the mixture by using simultaneous equations. Due to the incomplete separation of the N-COCH_3 peaks, accurate integration is not feasible and the application of the base line technique is useful.

2.4 2 CAFFEINE/CODEINE PHOSPHATE MIXTURE

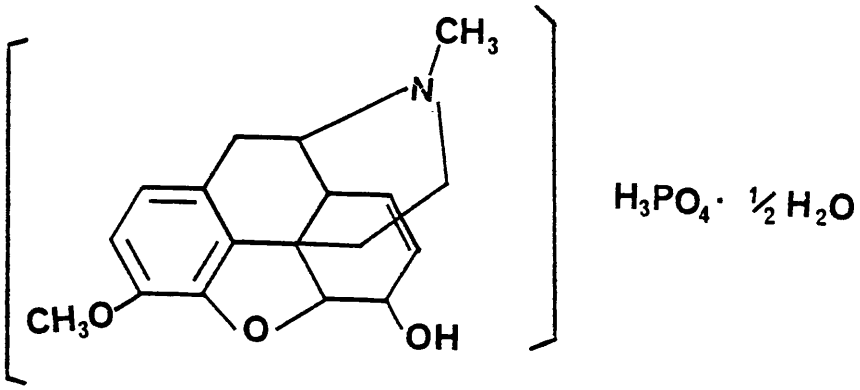
The OCH_3 and the N^7-CH_3 of codeine phosphate (2:35) and caffeine (2:34) respectively resonate close to each other δ3.73 and δ3.85 respectively in DMSO(d_6) but not so close as to permit the application of the base line technique (i.e. the molar ratios can be determined by direct integration). Addition of 3,4-dimethoxybenzoic acid (2:36) to the mixture of caffeine and codeine phosphate introduces two methoxy signals resonating at δ3.78 and δ3.80 ppm. This creates a suitable series of overlapping resonances for application



(2:34)



(2:36)



(2:35)

of the base line technique.

For the two results, two different curves were obtained when the separation (S) was plotted against the molar ratio, neither of which was linear. Figure 2:54 (page 188) shows the two graphs. The results are given in table 2:33(page 185). The caffeine/3,4-dimethoxybenzoic acid graph gave a concave curve whereas the codeine phosphate/3,4-dimethoxybenzoic acid graphs was convex. This is due to the fact that the 3,4-dimethoxybenzoic acid and caffeine or the 3,4-dimethoxybenzoic acid and codeine phosphate have completely different structures unlike that of benorylate and

Table 2:32 The base line technique for paracetamol/benorylate mixture

MOLAR RATIO: Paracetamol / Benorylate	Separation (S; mm)
0.486	13.3
0.620	15.6
0.836	19.8
1.151	22.8
1.443	27.3
1.700	30.3
1.953	32.8

Table 2:33 The base line technique for codeine/caffeine mixtures using 3,4-dimethoxybenzoic acid as the internal standard.

CAFFEINE		CODEINE PHOSPHATE	
MOLAR RATIO Caffeine / Int.standard	Separation (S; mm)	MOLAR RATIO Codeine phos. / Int.standard	Separation (S; mm)
1.165	32.0	1.134	28.3
1.003	28.4	1.002	23.6
0.660	22.8	0.538	16.9
0.5318	17.5	0.487	15.9
0.429	11.4	0.317	14.0

paracetamol (see general discussion, page 43).

FIG. 2:53 Base line technique for Benorylate/Paracetamol mixture

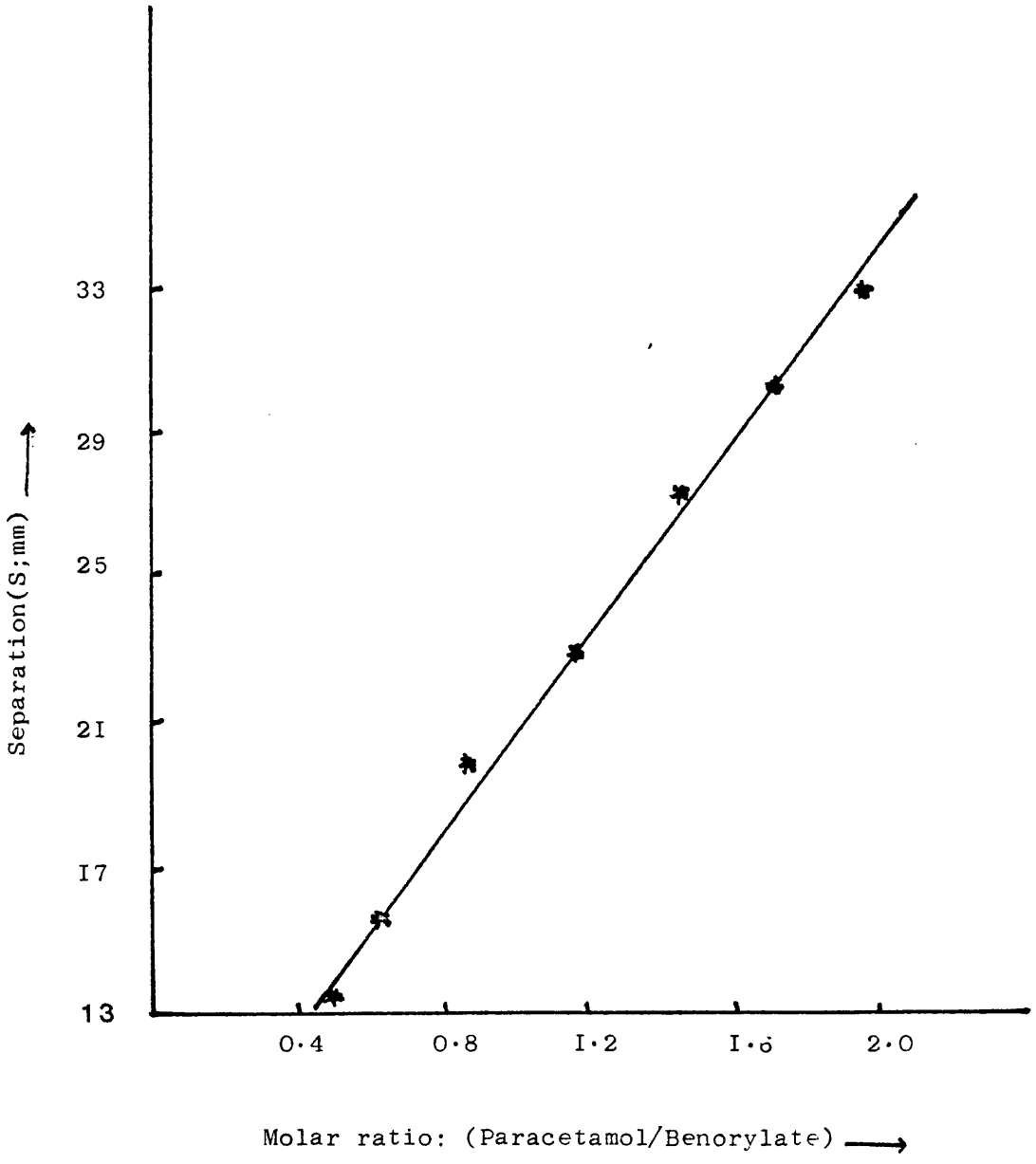
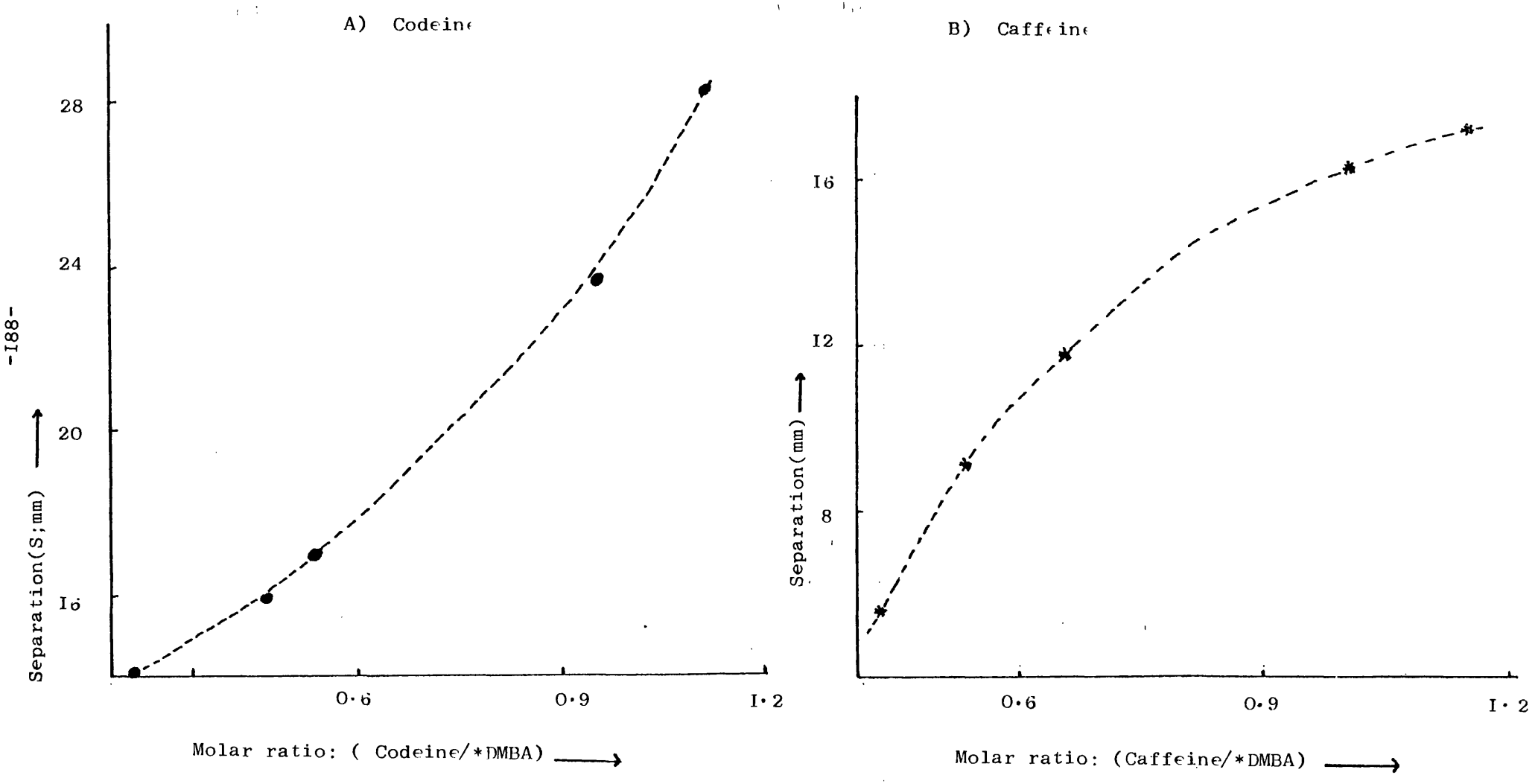


FIG. 2:54 Base Line Technique for Codeine/ Caffeine mixture



* DMBA = 3,4 dimethylbenzoic acid.

2.5 CONCLUSION

Chiral lanthanide shift reagents offer a simple, fast and accurate means of optical purity determination. For partially resolved resonance peaks the use of base line technique, chemical shift difference and peak height difference methods have been found suitable for optical purity or diastereomeric ratio determination. These techniques involve the use of a calibration curve and they give a correlation coefficient greater than 0.97.

The use of CLSR for optical purity determination is limited to compounds with few co-ordinating groups such as hydroxy and amino groups. For the optical purity of compounds with many such groups to be determined by the use of CLSR, derivatisation prior to the analysis is essential. Silylation has been found suitable in this respect.

2.5.1 Suggestions for further studies.

The base line technique is based on mathematical treatment (appendix). The equation derived involves two terms, a linear and quadratic. The quadratic term is significant, though the enantiomers and diastereomers studied gave linear relationship. Further investigation is needed to establish the relationship between structure and the technique.

To achieve base line separation of chromatograms, in HPLC or GLC, alterations in the column conditions such as the flow rate, solvent systems, packing materials etc. is the normal procedure. This often makes analysis lengthy. Application of the base line technique may solve this problem.

2.6 EXPERIMENTAL

Except where specified, all nmr spectra were recorded on a Perkin Elmer R12B instrument operating at 60 MHz and a probe temperature of $37^{o} \pm 1^{o}C$. TMS was used as the internal reference compound.

Infra red spectra were obtained on liquid films or as KCl discs (for solids).

Mass spectra were obtained on M.S. 12 instrument at 70 eV.

Elemental analysis were performed by the Butterworth Laboratories Ltd., Middlesex.

Melting points were recorded on a Gallenkamp melting point apparatus No. 889339.

NMR solvents, CCl_4, $CDCl_3$, C_6D_6 and DMSO(d_6) were dried over a molecular sieve (4A).

Lanthanide shift reagents, $Eu(fod)_3$, $Pr(fod)_3$, $Eu(facam)_3$ and $Pr(facam)_3$ were purchased and stored over P_2O_5 in a desiccator.

Chemicals used were obtained from the following sources:

N, N-Bis-(trimethylsilyl)trifluoroacetamide was purchased from Aldrich Chemical Co. Ltd. and stored in a refrigerator.

(±), (-) and (+)-ephédrines and pseudoephedrines as their hydrochlorides* and sulphates*, and (-) ephedrine base were supplied by Lake and Cruickshank Ltd., Manufacturing Chemists.

Propranolol (l, d and dl) as their hydrochlorides* were supplied by ICI Ltd.

(±)-Salbutamol, R(-) salbutamol and S(+) salbutamol were supplied by Allen and Hanburys Ltd., London.

Tetramisole -, Dexamisole- and Levamisole- hydrochlorides* were supplied by Janssen Pharmaceutic, N.V. Beerse/Belgium.

Racemic ibuprofen, d-ibuprofen and l-ibuprofen were supplied by the Boots Company Ltd., Nottingham.

(±)-Naproxen, (+) naproxen and (-) naproxen, supplied by the Syntex Laboratories Inc., California.

(±)-Ketoprofen and (+) ketoprofen, supplied by May and Baker.

(±)-Fenoprofen and (-) fenoprofen calcium salts, supplied by Lilly Research Centre Ltd.

Atropine and hyoscyamine were purchased from Sigma Chemical Co. Ltd., Poole.

DL-Dopa and L-Dopa were purchased from Aldrich Chemical Co. Ltd., Gillingham.

Quinine $3H_2O$ and Quinidine $2H_2O$, caffeine, codeine phosphate and paracetamol were purchased from Evans Medical Ltd., Bristol.

* The bases were obtained from their proton salts by dissolving them in

water, basifying with NaOH (20%) and extracting with ether or chloroform. The organic layer was dried over anhydrous sodium sulphate and then removed. The residue was dried *in vacuo* at a temperature not exceeding 50°C.

2.6.1 Determination of LIS:

A solution (*Ca* 0.2 - 0.4 M) of the sample in the appropriate solvent (0.7 ml) was prepared. The nmr spectrum was recorded. $Eu(fod)_3$ and $Pr(fod)_3$ in 10 - 20 mg quantities were added incrementally and the spectrum recorded again after each addition. The LIS of the appropriate signals were measured and plotted against the molar ratio (Reagent / Substrate).

2.6.2 Determination of Differential Shift ($\Delta\Delta\nu$)

The above procedure (section 2.6.1) was repeated with the racemic material and employing the chiral shift reagents $Eu(facam)_3$ and $Pr(facam)_3$.

2.6.3 Optical purity determination

2.6.3.1 For ephedrine, pseudo ephedrine, propranolol, tetramisole, ibuprofen, naproxen, fenoprofen and atropine/hyoscyamine.

Different quantities of the l and d isomers[+] (or the racemic and any of the pure isomers available) were mixed to give different optical purities but approximately the same final concentration. Each mixture was dissolved in the appropriate solvent[*] (0.7 ml) and a

* Details of solvents, CLSRs and molar ratios are given in the appropriate tables

\+ For propranolol, the *N*-acetyl derivative (2:7) was employed. For fenoprofen, the methyl ester derivative (2:20) was used. For atropine/hyoscyamine, tropic acid methyl ester (2:22) was used. (See page 194 for the synthesis of the various derivatives.)

quantity of the *CLSR added to yield the appropriate *molar ratio ($Eu(facam)_3$ / total substrate). The nmr spectra of the various resulting solutions were recorded under the same instrumental conditions. The base line (S), the chemical shift difference (δv) and the peak height difference (δh) of the analytical signals were measured and plotted against the appropriate parameter. Where suitable, integrals of the analytical signals were recorded.

2.6.3.2 Salbutamol

Various quantities of R(-)salbutamol and S(+)salbutamol were mixed to obtain different enantiomeric mixtures. In each case the total weight of salbutamol taken was 57 - 61 mg. BSTFA (0.4 ml) was added to each mixture and the temperature then maintained at 130°C for 10 - 15 mins. The excess BSTFA was removed *in vacuo* and the product treated with an amount of $Eu(facam)_3$ which would give a molar ratio of 1.602 when benzene (d_6) was used or 1.19 when carbon tetrachloride was used as the solvent. The nmr spectra of all solutions were recorded and the base line technique, chemical shift difference and peak height difference methods used for the analysis employing the OCH_2 signal at δ4.6 - 5.4 ppm.

2.6.3.3 Ketoprofen

Different quantities of racemic ketoprofen and the d-isomer were methylated as described (page 195). The methyl ester mixtures were dissolved in CCl_4 to give a total concentration of about 0.182 M in each case. A calculated amount of $Eu(facam)_3$ was added to each mixture in CCl_4 to give a molar ratio ($Eu(facam)_3$/ketoprofen methyl ester) of 0.406. The nmr spectrum of each solution was recorded.

The C-CH_3 signal at δ1.9 - δ2.15 ppm was expanded (scale expansion at 100 Hz) and the base line technique and the chemical shift difference method applied for the analysis.

2.6.3.4 Dopa

Various quantities of the L- and DL-Dopa methyl ester were mixed together to obtain different enantiomeric ratios. These were used to prepare the silyl derivative of dopa methyl ester (page 197). The products were dissolved in CCl_4. A calculated amount of $Eu(facam)_3$ was added to each mixture to give a molar ratio of 0.842. [The calculation of the molar ratio was based on the weight (0.06g) of the dopa methyl ester]. The nmr spectra of the various mixtures were recorded. The $COOCH_3$ resonance peaks at δ5.80 - 6.1 ppm were used for the analysis employing the base line technique, chemical shift difference and peak height difference methods.

2.6.4 Synthesis of Derivatives

2.6.3.1 *N*-acetylpropranolol

Freshly distilled acetic anhydride (0.3 mls) was added to propranolol (0.85 g) dissolved in dried tetrahydrofuran (5 ml). The mixture was stirred overnight. The solvent was removed *in vacuo* and the residue dissolved in chloroform (20 ml.). The organic layer was washed with N/10 HCl (10ml), and then with N/10 NaOH (10 ml). The chloroform was removed and the residue (an oil) crystallized from petroleum ether (b.p. 40 - 60°C)/ether (1:3) mixture. The sample was dried, and then recrystallized from ether yielding *N*-acetylpropranolol (0.45/g; 45.6%), m.p. 103.9 - 104.2°C.

ν_{max}: 2950, $\overset{1690}{\backslash}$1603, 1470, 1385 cm^{-1}.

Found: C, 70.8; H, 7.8; N, 4·8%.

$C_{18}H_{23}NO_3$ requires: C, 71.7; H, 7.6; N, 4.7%.

The nmr spectrum is shown in figure 2:12 (page 86).

2.6.4.2 $(O\text{-}TMS)_3$ Salbutamol.

Salbutamol (0.06g) was treated with excess of BSTFA (*ca* 0.4 ml) in a stoppered flask (5 ml) and the temperature maintained at 130°C for 10 - 15 mins. The excess BSTFA was removed *in vacuo* at about 50°C. The oil remaining was distilled. The fraction b.p. 140 - 142°C/0.8 mm Hg. was collected. ν_{max}: 3,000, 1620, 1508, 1262, 860 cm^{-1}. M/e (%); 457 (2), 442 (5.7), 370 (34), 368 (98), 192 (3.4), 148 (18), 147 (100).

Found: C, 57.9; H, 9.9; N, 3.7; Si, 18.0%.

$C_{22}H_{45}NO_3Si_3$ requires C, 58.3; H, 9.9; N, 3.1; Si, 18.5%.

The nmr spectrum is shown in figure 2:16 (page 97).

2.6.4.3 Ketoprofen methyl ester

Ketoprofen (0.05 g) was dissolved in methanol (*ca* 20 ml) containing conc. HCl (*ca* 1 ml). The mixture was refluxed for 1 hr. The excess methanol was removed *in vacuo* and the liquid residue, Ketoprofen methyl ester, used for the analysis. ν_{max}: 1740, 1690, 1590, 1500 cm^{-1}.

The nmr spectrum is shown in figure 2:32A (page 140).

2.6.4.4 Fenoprofen methyl ester

The calcium salt (0.5 g) was dissolved in hot methanol (*ca* 40 ml) and the mixture acidified with conc. HCl (*ca* 1 ml). The solution was concentrated *in vacuo*, and the residue, fenoprofen methyl ester,

as a liquid, was used for the analysis. ν_{max}: 1735, 1590, 1500, 1250 cm^{-1}.

The nmr spectrum is given in figure 2:35A (page 147).

2.6.4.5 Tropic acid methyl ester

2.6.4.5.1 dl-tropic acid methyl ester

*Atropine (0.6 g) was dissolved in ethanolic sodium hydroxide solution (20%, 40 ml) and refluxed for 1 hr. The reaction mixture was allowed to cool and extracted with chloroform (3 x 20 ml). The organic layer was discarded after washing with water (3 x 4 ml). The bulked aqueous layer was acidified with conc. HCl (1 ml) and extracted with chloroform (3 x 20 ml). The combined chloroform extract was dried (Na_2SO_4) and removed *in vacuo*. The residue was dissolved in methanol (30 ml) containing conc. HCl (1 ml) and the mixture refluxed for about 1 hr. The methanol was removed *in vacuo*. The dl-tropic acid methyl ester (0.23 g, 62.6%), m.p. 35°C crystallized as fine needles from petroleum ether (b.p. 60 - 80°C), m.p. 37° - 38°C. (lit.[159] 36.5 - 37.5°C). ν_{max}: 3200 - 3600, 2960, 1750, 1500 cm^{-1}.

The nmr spectrum is shown in figure 2:39 (page 157). Addition of $Eu(facam)_3$ at a molar ratio ($Eu(facam)_3$/dl-tropic acid methyl ester) of 1.00 gave two singlets at δ6.8 and δ7.8 ppm in CCl_4 (3H, 2S, $COOCH_3$).

2.6.4.5.2 l-tropic acid methyl ester

l-hyoscyamine (0.5 g) was refluxed in N/10 HCl (30 ml) for 2 hr.

* Hyoscyamine gives similar results (see page 155).

The reaction product was allowed to cool and extracted with ether (60 ml). The ether was dried (Na_2SO_4) and removed *in vacuo*. The residue was dissolved in methanol (30 ml) containing conc. HCl (1 ml) and the mixture refluxed for about 1 hr. The methanol was removed *in vacuo*. The l-tropic acid methyl ester (0.18 g, 59.1%), m.p. 3.6-37.5°C. ν_{max}: 3200 - 3600, 2960, 1750, 1500 cm^{-1}. Addition of $Eu(facam)_3$ at a molar ratio of 1.00 ($Eu(facam)_3$/(-tropic acid methyl ester) gave a singlet at δ7.8 ppm in CCl_4 (3H, S, $COOCH_3$).

2.6.4.6 Dopa methyl ester

Dopa (1.0 g) was dissolved in methanol (*ca* 40 ml) containing conc. HCl (1 ml). The mixture was refluxed for 1 hr and the methanol removed *in vacuo*. The product was crystallised from aqueous ethanol yielding dopa methyl ester as, (0.56 g, 52.5%), m.p. 103 - 105°C. ν_{max}: 3200 - 3600, 1745, 1490 cm^{-1}. δ(CCl_4): 2.65 - 3.00 (2H, m, CH_2), 3.40 - 3.75 (1H, m, C*H*), 3.70 (3H, S, $COOCH_3$), 6.72 (3H, S, Ar-*H*).

Found: C, 57.5; H, 5.9; N, 6.5%.

$C_{10}H_{13}NO_4$ requires: C, 56.9; H, 6.2; N, 6.6%.

2.6.4.7 $(TMS)_3$-Dopa methyl ester

Dopa methyl ester (*ca* 60 mg) and BSTFA (0.5 ml) were heated at 160°C for 10 mins. The excess BSTFA and the trifluoroacetamide were removed. The product was used immediately for the enantiomeric ratio analysis.

δ(CCl_4); 0.02 (9H, S, $Si(CH_3)_3$, 0.30 (18H, S, 2X-$Si(CH_3)_3$, 2.60 - 2.95 (2H, m, -CH_2), 3.30 - 3.69 (1H, m, C*H*), 3.62 (3H, S, $COOCH_3$), 6.64 (3H, S, Ar-*H*).

2.6.5 Determination of diastereomeric ratios of Quinine/Quinidine and Ephedrine/pseudo-ephedrine.

Different quantities of quinidine and quinine or ephedrine and pseudo-ephedrine were mixed together in $CDCl_3$ or C_6D_6 respectively to obtain different diastereomeric ratios but the same total concentration (see tables 2:30A, page 172 and 2:31, page 175). The nmr spectrum of each mixture was recorded and the chemical shift difference (δv), the separation (S) and the peak height difference (δh) of the analytical peaks (OCH_3 for quinine/quinidine and N-CH_3 or C-CH_3 for ephedrine/pseudo-ephedrine) measured. The values obtained were plotted against the appropriate parameters (see figures 2:47 to 2:51, pages 177&181). For the quinine and quinidine mixture, $Pr(fod)_3$ (32 mg) was added to each mixture and the procedure repeated (see table 2:30B, page 173).

2.6.6 Base line technique for dissimilar molecules

2.6.6.1 Benorylate/paracetamol mixture

Different amounts of benorylate and paracetamol were dissolved in $CDCl_3$/DMSO(d_6) (2:1, 0.7 ml) to give different molar ratios (see table 2:32, page 185). The nmr spectrum of each mixture was recorded. The analytical region, δ2.0 - 2.1 ppm, due to the N-CH_3 peaks of the two compounds were expanded on a 100 Hz scale and the separation (S) recorded and plotted against the molar ratio (paracetamol/benorylate).

2.6.6.2 Caffeine/codeine phosphate mixture

Different quantities of caffeine, dimethoxybenzoic acid (DMBA) and codeine phosphate were dissolved in DMSO(d_6);(0.7 ml) to give

different caffeine/DMBA and codeine phosphate/DMBA - molar ratios (see table 2:33, page 135). The nmr spectrum of each mixture was recorded. The analytical region , δ3.7 - 3.9 ppm, due to the OCH_3 of codeine phosphate, N^7-CH_3 of caffeine and 2(OCH_3) of DMBA, was expanded on a 100 Hz scale. The separation (S) due to caffeine/DMBA at δ3.85 - 3.80 ppm and that due to codeine phosphate/DMBA at δ3.73 - 3.78 ppm were measured and plotted against the molar ratios.

PART 3

3. NMR QUANTITATIVE ANALYSIS OF PHARMACEUTICALS

3.1 GENERAL DISCUSSION

Many pharmaceutical preparations have been assayed successfully by nmr. The method involves the addition of an internal standard to the sample and subsequent extraction with a solvent. The appropriate analytical peaks are integrated after the nmr spectrum has been recorded. The weight (W_t) of the sample is then calculated from the following general formula:

$$W_t = \frac{E_T \cdot I_T \cdot W_S}{E_S \cdot I_S}$$

where E_T is the nmr equivalent weight of the sample

E_S is the nmr equivalent weight of the standard

I_T is the integral of the sample analytical peak(s)

I_S is the integral of the standard analytical peak(s)

W_S is the weight of the standard taken.

Except where indicated this was the method of calculation adopted for all assays discussed.

The internal standards employed and their equivalent weights are listed in table 3:1A (page 201). In some of the assays, the pure samples of the active ingredients were used as internal standards for two reasons:

(1) where no available internal standard was suitable and

(2) to demonstrate the usefulness of the chemical itself as internal standard.

In addition to the nmr method, the official methods of analysis were also performed for comparison. A summary of the results are given in table 3:1B (page 202). As seen in this table, the nmr and

Table 3:1A Internal standards employed for the analysis of the pharmaceuticals

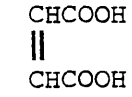
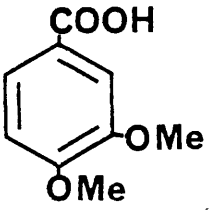
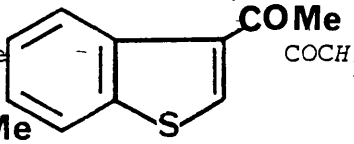
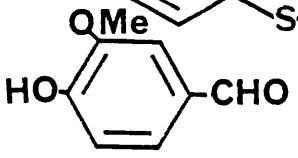
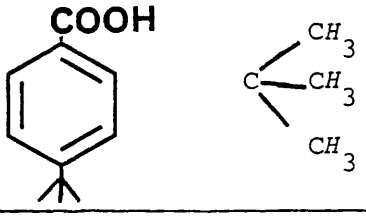
Name	Structure	Analytical group	Resonance position (δ; ppm)	Equivalent weight
Maleic acid		$CH{=}CH$	6.35	58.04
3,4-dimethoxybenzoic acid		$2(OCH_3)$	3.80	30.36
3-Acetobenzthiophene		$COCH_3$	2.6	58.72
Vanillin		CHO	9.8	152.16
p-tert. butylbenzoic acid		$C(CH_3)_3$	1.29	19.80

Table 3:1B Summary of results obtained from nmr and official methods

Formulation	Active ingredient	Av.% Labelled strength		Coefficient of variation (%)	
		nmr	Official	nmr	Official
Panadol	paracetamol	100.8	101.2	2.47	0.67
Brufen tablets	ibuprofen	99.7	99.0*	7.89	0.76
Brufen suspension		101.0	100.5*	1.75	0.83
Ephedrine HCl	-	100.9	100.0	1.03	0.03
Pseudo-ephedrine HCl		100.4	-	2.28	-
Butazolidine	phenylbutazone	102.3	100.9	0.78	0.57
Alka					
Orudis	ketoprofen	97.1	97.6*	1.65	1.07
Tanderil	oxyphenbutazone	97.3	98.5	1.32	0.72
Inderal	propranolol HCl	96.4	98.8	2.21	0.52
Indocid	indomethacin	104.6	100.9	2.64	0.72
Naprosyn	naproxen	97.4	100.2*	1.91	0.51
Benoral tablets	benorylate	101.4	100.5*	4.78	0.77
Benoral suspension		110.4	99.6*	5.67	0.73
Equagesic	meprobamate	102.6	101.0	0.80	0.40
	aspirin	97.4	98.0	1.21	0.53
	ethoheptazinecitrate	96.4	98.9	2.38	0.55
Codis	aspirin	105.2	101.6	2.38	0.44
	codeine phosphate	94.7	94.5	2.00	0.79
Cafadol	paracetamol	98.0		1.79	
	caffeine citrate	104.5		3.76	
Parahypon	paracetamol	96.6		2.36	
	caffeine	94.2		2.24	
	codeine phosphate	- 1.6		1.14	

* UV method (unofficial)

the official methods have comparable accuracy in most cases. One clear exception was "Benoral" suspension where the average percent labelled strength (110.4) was high as compared with that of the other method (99.6).

The nmr method had four advantages: simplicity, speed, sample size and specificity.

Simplicity: In most cases the nmr method was simpler than the official methods, except those cases where a UV analysis was possible.

Speed: NMR offers a rapid method of analysis. The maximum duration for a single analysis was 40 minutes including sample preparation. In most cases this is only a fraction of the time required by official methods. In this respect, nmr is comparable to UV analysis. It has a clear advantage over the other official methods.

Sample size: The quantity of sample required for nmr analysis is small. Concentrations of about 10% are generally required[18] for good nmr spectra, but since the volume of solvent required is small, this concentration is obtained with a small amount of the sample.

Specificity: The nmr spectrum often reveals the presence of any impurities in significant concentration and any changes in the structure of the compound under investigation by the appearance of extra resonance peaks or disappearance of a known peak. It is often possible to identify the impurities, and in this respect, nmr quantitative analysis is a useful quality control method.

The nmr method was less precise than the existing methods (see table 3:1B, page 202). A higher coefficient of variation was obtained in all the analysis compared with the existing methods. Since

most of the published work on nmr quantitative analysis claim low standard deviations[49], the high value obtained in this work might be due to instrumental factors.

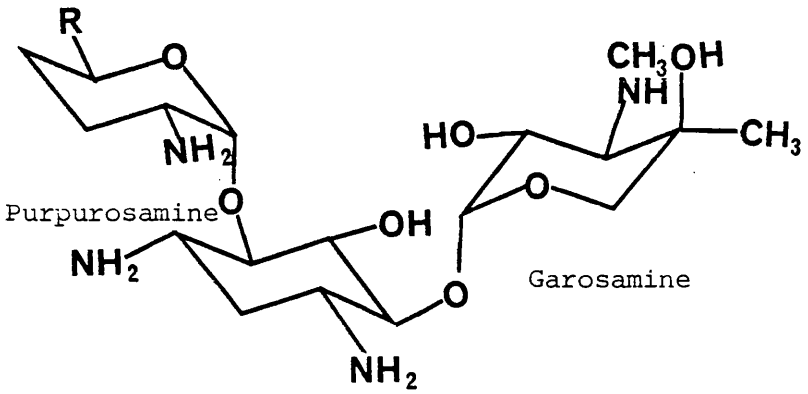
NMR has long been noted as unsuitable for assay of low dosage pharmaceuticals[2]. The assays of 'Codis' (section 3.4.2, page 264), 'Cafadol' (section 3.4.3, page 269) and 'Parahypon' (section 3.4.4 , page 269) clearly demonstrate the use of nmr for low dosage drugs. Addition of a known weight of the analar drug to the analytical sample increases the nmr signal sensitivity of the sample and therefore affords measurable integrals. The codeine phosphate content of 'Codis' is 8 mg per tablet. The nmr analysis gave 94.7% labelled strength which compared favourably with the results obtained by the official method (94.5%). Satisfactory results were also obtained for the caffeine citrate and caffeine content in 'Cafadol' and 'Parahypon' respectively.

One problem with nmr quantitative analysis is the possible inteference from excipient materials (see section 1.2, page 16). In this work, there was only one clear case where excipient resonance peaks interfered with the analytical peaks which precluded the nmr assay (see section 3.4.5, page 273).

The success of nmr quantitative analysis depends critically on the method of measurement of the analytical signals. When peak height method was compared with integration, the former method was found less accurate than the latter. An error as high as 10.74% was obtained for peak height measurement and 0.82% for integration in the assay of 'Panadol' (section 3.3.1, page 217). In the same

analysis, peak height measurement was found less precise (coeff. var. 5.21%) than integration (coeff. var. 2.47%). Similar results were obtained for gentamicin analysis (see table 3:3, page 210).

3.2 GENTAMICIN C



Deoxystreptamine

(3:1)

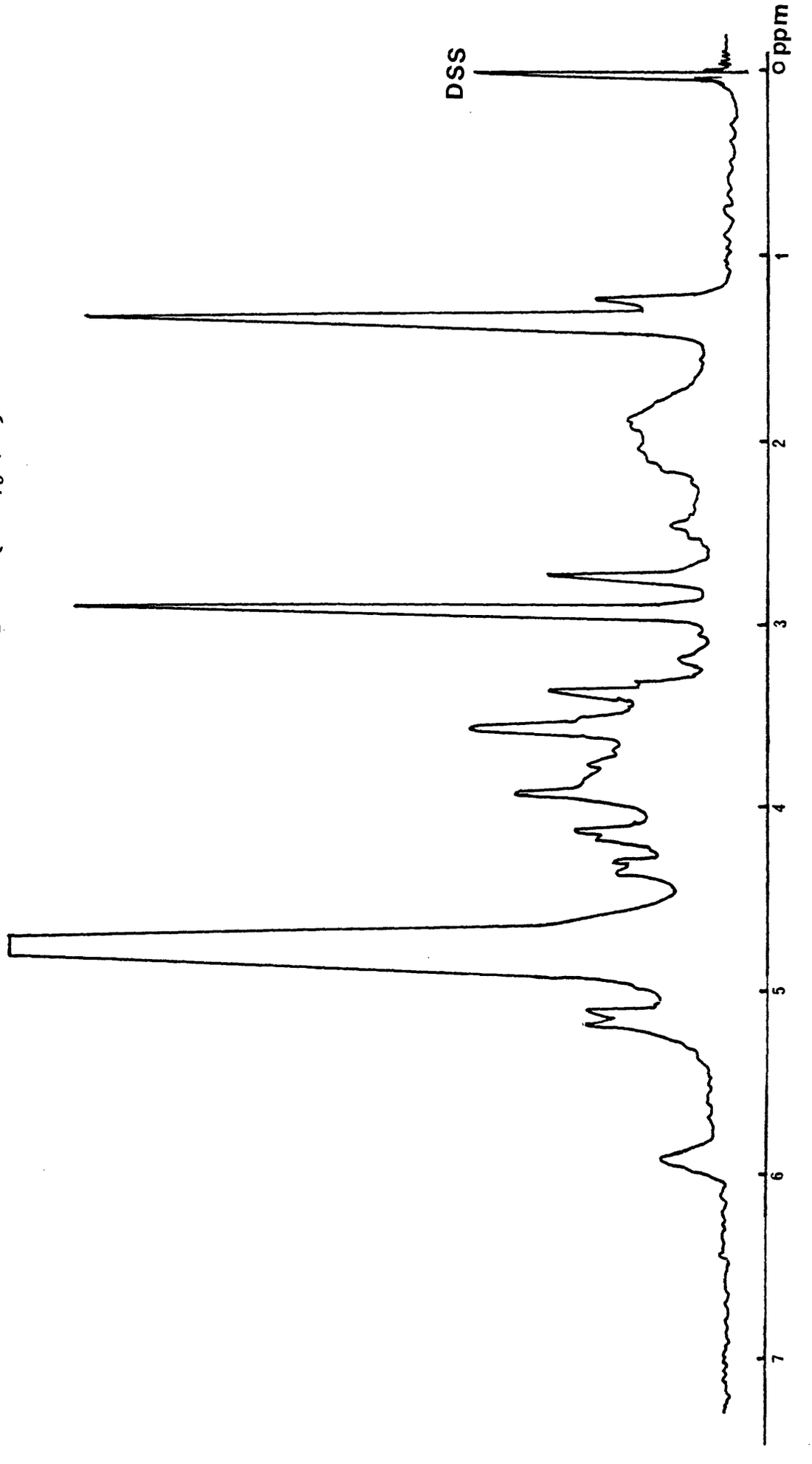
R = $-CH(CH_3)NHCH_3$ ... Gentamicin C_1

R = $-CH_2NH_2$ ... Gentamicin C_{1a}

R = $-CH(CH_3)NH_2$ ... Gentamicin C_2

Gentamicin C(3:1) is a broad spectrum, basic deoxystreptamine containing antibiotic produced by Micromonospora species[161,162]. It is a mixture of three major components designated C_1, C_2 and C_{1a}. Analysis indicates that C_1 forms about 35 - 50%, C_2 forms about 30 - 45% and C_{1a} forms about 10 - 20% of the total Gentamicins[161,164]. They have, however, almost the same biological activity[161,164]. Separation and differentiation of this gentamicin complex has been effected by chromatographic techniques[164-166]. Assay procedures for the determination of the total Gentamicins and the individual components are based on microbiological assay and paper chromatographic techniques[167,168,170]. NMR has also been adopted as a limit test to monitor the proportions of the main components[168,178].

FIG. 3:1 Gentamicin Sulphate in D_2O (19.8% w/v)



Recently a comparison of the three methods - microbiological, chromatographic and nmr - has shown that nmr is the most useful method for evaluating the composition of gentamicin sulphate[169]. The standard erros obtained for gentamicin C_1 by the three methods were ± 5.61, ±5.32 and ±1.38 respectively.

The nmr method[168] is a limit test based on the *N*-methyl ratio of the peak at δ2.75 to the peak at 2.95 (see table 3:2). Therefore it does not give the actual amount of gentamicin present. In this report an nmr method of assay is described for the total gentamicins, as well as the individual components (C_1, C_2 and C_{1a}). It is based on integration of the signals at δ1.25 - 1.35, δ2.75 and δ2.95 (table 3:2), using maleic acid as internal standard and D_2O as solvent.

Table 3:2 Assignment of the analytical peaks

Position of signal (δ, ppm)	Assignment	Gentamicin
2.95	N-C*H*$_3$	Total
2.75	N-C*H*$_3$	C_1
1.35	C-C*H*$_3$	Total
1.25	N-CH-C*H*$_3$	C_1+C_2

Integration of the signal at δ2.95 gives an area indicative of the total gentamicins since the resonance is due to the N-C*H*$_3$ of the Garosamine moiety which is common to all three components. Similarly, the signal at δ1.35 is due to the C-C*H*$_3$ of the Garosamine ring and

hence represents the total gentamicins. Integration of the latter signal is, however, not feasible because of signal overlap (figure 3:1, page 207). The signal at δ1.25 represents both C_1 and C_2 (table 3:2), and therefore total integration of the two signals at δ1.25 and 1.35 represents the total gentamicins plus C_1 and C_2. Integration of the signal at δ2.75 represents C_1.

3.2.1 Assay of total gentamicins

The assay procedure was applied to three separate gentamicin samples. Two of the samples were gentamicin sulphate powder and the third was gentamicin injection. Ten determinations were made on each sample. The N-C*H*$_3$ signal at δ2.95 and the maleic acid olefinic proton signal at δ6.35 were utilised for the analysis. Peak height measurement was employed on one sample for comparison. A summary of the results is shown in table 3:3 (page 210).

The equivalent weight of the total gentamicins (E_T) was obtained as shown below:

$$E_T = \frac{\text{Total M. Wt. of the gentamicins}}{\text{Total no. of protons giving the signal at } \delta 2.95}$$

$$\therefore \frac{477 + 463 + 449}{9} = \underline{154.33}$$

The ratios of the individual components were approximated to be 33½ % each.

The results obtained from the peak height measurement clearly shows that this method is not satisfactory for the assay of gentamicin.

Table 3:3 Summary of total gentamicins assay results

Sample and Batch No.	Labelled strength	Mean potency found		Standard deviation		Coefficient of variation (%)	
Gentamicin sulphate powder No. 60901	58.3%,w/w	56.4%, w/w		2.46		4.4	
Gentamicin Injection No.11OE	80.10 mg/2ml	71.10 mg/2ml		2.99		4.2	
		by Integration	by Peak height	by Integration	by Peak height	by Integration	by Peak height
Gentamicin sulphate powder No. 61101	58.3%,w/w	55.6%, w/w	31.0%, w/w	2.43	2.56	4.4	8.3

3.2.2 Assay of the individual components of gentamicin

The calculation for the individual gentamicins was based on the following:

Table 3:4

Signal	Position of signal	Gentamicin
A	δ2.95	Total
B	δ2.75	C_1
E	δ1.35 - 1.25	Total + C_1 and C_2

From Table 3:4,

$$C_1 + C_2 = \text{signal E - signal A} = D$$

$$C_2 = D - B$$

$$C_{1a} = A - D \text{ or } 2A - E$$

$$\text{Wt. of } C_1 = \frac{159 \times B \times W_s}{E_s \times I_s}$$

$$\text{Wt. of } C_2 = \frac{154.33 \times (D-B) \times W_s}{E_s \times I_s}$$

$$\text{Wt. of } C_{1a} = \frac{149.67 \times (2A-E) \times W_s}{E_s \times I_s}$$

where 159, 154.33 and 149.67 are the equivalent weights of C_1, C_2 and C_{1a} respectively.

The integrals of signals A, B and E were recorded and individual weights established. The results are given in table 3:5, page 212 .

Table 3:5 Assay of the individual components of Gentamicin

Sample: Gentamicin sulphate powder Batch No. GMC 4-X-6007

Expt. No.	Wt. of Maleic acid (mg)	Wt. of Gentamicin sample (mg)	Av. integrals (cm) I_s	A	B	E	Wt. of total Gentamicin found (mg)	% w/w of the individual Gentamicins found C_1	C_2	C_{1a}	% w/w of total Gentamicins found
1	39.0	89.2	5.56	2.56	0.90	3.89	47.5	36.57	17.55	45.88	53.16
2	43.0	83.0	5.60	2.04	0.69	3.00	41.3	34.84	13.72	51.44	49.87
3	41.6	83.6	6.03	2.29	0.79	3.67	42.0	35.55	25.73	38.72	50.26
4	42.9	89.4	4.86	2.18	0.71	3.13	50.7	33.62	11.62	54.76	56.69
5	48.0	84.8	6.37	2.50	0.79	3.63	49.7	32.73	13.99	53.28	58.58
6	42.7	85.7	5.05	2.03	0.62	2.97	45.3	31.62	16.05	52.33	52.85
7	36.5	81.2	5.77	1.76	0.47	2.53	45.3	27.68	17.34	54.98	55.84
8	43,4	87.9	5.65	2.21	0.79	3.17	44.9	36.94	7.51	55.56	51.07
9	36.4	81.0	4.31	1.97	0.70	2.77	44.0	37.43	5.16	57.41	54.32
10	43.7	81.1	4.44	1.48	0.45	2.22	38.9	31.46	19.27	49.27	47.91
							MEAN	33.84	14.79	51.36	53.24
							S. Deviation	2.89	5.91	5.46	2.97
							Coeff. variation	8.55%	39.97%	10.63%	5.58%

% w/w found = 53.2

Labelled strength = 55.9% w/w

The results for the average % w/w obtained for the total gentamicins was below the labelled strength. To investigate this, known quantities of the individual gentamicins were mixed together and assayed. The results are expressed in table 3:6 (page 215).

The results obtained for the individual components were inconsistent. Moreover they were contrary to that expected (page 206). When the spectrum of gentamicin C_1 alone was recorded, it was observed that signals A (δ2.95) and B (δ2.75) were not equal both in height and in area (integral). The two signals should be equal in every respect for gentamicin C_1. This may account for the low results obtained for C_1.

From a consideration of structure twice the integral for signal A should equal the integral for signal E (δ1.35 - 1.25) for pure C_1 and pure C_2, but, again, this was not observed. Rather 2A > E. This may account for the low value of C_2 and high value of C_{1a}. It is considered that these results may reflect interference from other minor components in the gentamicin mixture[169].

In view of these findings it was thought reasonable to investigate whether there was a constant ratio between signals A and B, and that of 2A and E, in different experiments using C_1 and C_2, and a mixture of the two. The results are shown in table 3:7, page 216. It is clear that there is no constant ratio between signals A and B, and between signals E and A. This might be due to instrumental factors. Since no constant ratio was obtained in either case, the method is not applicable to the assay of individual

components of gentamicin C.

3.2.3 CONCLUSION

Though the indivdual components of gentamicin C could not be assayed successfully, the method is not without merit. The total gentamicins may be assayed successfully by this method. Under proper conditions of integration and measurement of integrals, the method gives 100.5% accuracy with 4.4% variation. The assay can be completed in about 30 mins. The biological assay method takes up to 18 hours, and the ion exchange chromatographic method takes 14 hours per sample [169].

Table 3:6 Total gentamicin assay using mixture of pure C_1, C_{1a} and C_2

Wt. of Maleic acid (mg)	Total wt. of Gentamicin taken (Wt; mg)	Av. integrals(cm) I_s	Av. integrals(cm) $I_T(\delta 2.95)$	Wt. of Gentamicin obtained (Wo; mg)	$\frac{Wo}{W_t}$ x 100 (%)
40.8	48.4	5.06	2.11	45.2	93.4
40.2	52.7	4.98	2.47	53.1	100.7
40.7	35.9	4.99	1.63	35.4	98.6
41.1	45.6	5.12	2.11	45.0	98.8
39.8	60.1	4.87	2.90	63.1	105.0
40.3	28.5	4.96	1.43	30.9	107.5
40.2	104.1	4.96	4.74	102.1	98.0
40.1	36.0	4.93	1.70	36.8	102.3
				MEAN:	100.5%
				Stand. Deviation	4.4
				Coefficient of variation	4.4%

Solvent: D_2O (0.5 ml)

Table 3:7 Results for the ratios between signals E and 2A; and B and A.

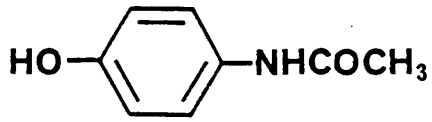
Gentamicin sample	Wt. taken (mg)	Ratio: $\frac{E}{2A}$	Ratio: $\frac{B}{A}$
C_1	36.0	0.825	0.673
C_1	28.5	0.823	0.676
C_1	46.5	0.802	0.695
C_1	25.4	0.777	0.665
C_1	19.1	0.806	0.677
C_1	29.3	0.851	0.769
$C_1 + C_2$	52.7	0.821	
$C_1 + C_2$	39.5	0.820	
C_2	16.0	0.773	
C_2	25.8	0.687	
C_2	60.1	0.737	
C_2	45.6	0.761	

3.3 PHARMACEUTICAL PREPARATIONS CONTAINING ONE ACTIVE INGREDIENT

3.3.1 'PANADOL'

'Panadol' is a tablet preparation with paracetamol (500 mg) as the active ingredient. It is used as an analgesic and antipyretic.

Paracetamol (2:32) shows a sharp singlet at δ2.01 ppm due to the $NCOCH_3$ group. On the basis of the paracetamol spectrum and the availability of material, 3,4-dimethoxybenzoic acid was selected as the internal standard. Paracetamol and 3,4-dimethoxybenzoic acid were only sparingly soluble in $CDCl_3$ but readily soluble after addition



(2:32)

of a few drops of DMSO(d_6). A mixture of $CDCl_3$/DMSO(d_6) (1:3) was therefore used for the analysis. For comparison, both integration and peak height were employed for the measurement of the resonance peaks. The results are given in table 3:8, page 218.

The official[153] method of assay for paracetamol tablets was followed and the results compared with those obtained for the nmr method. The results are given in table 3:9, page 219 . The official method involves extraction of paracetamol into NaOH followed by measurement of the extinction of the resulting solution at 257 nm.

Using integration, the average weight of paracetamol obtained per tablet (504.1 mg) compares favourably with the result obtained by the official method (506.2 mg). Using the peak height measurement

Table 3:8 Results for the nmr assay of paracetamol tablets

Av. Wt. of a tablet = 585.9 mg

Exp. No.	Wt. of internal standard (mg)	Wt. of powdered tablets taken (mg)	Av. integrals (cm) I_s	Av. integrals (cm) I_T	Av. peak heights (cm) H_s	Av. peak heights (cm) H_T	+Actual wt. of paracetamol (mg)	Wt. of paracetamol found (mg) by integration	Wt. of paracetamol found (mg) by peak height
1	187.5	249.7	1.38	0.93	7.75	5.00	213.1	209.8	200.8
2	198.2	248.2	1.30	0.84	6.85	3.90	211.8	212.6	187.3
3	196.2	248.0	1.49	1.00	7.40	4.10	211.6	21.92	180.5
4	201.9	248.9	1.40	0.91	7.50	3.90	212.4	218.2	174.3
5	197.6	250.4	1.39	0.90	5.90	3.80	214.8	213.6	211.3
6	145.0	244.1	1.32	0.82	6.50	3.75	208.3	199.5	186.8
7	197.6	248.6	1.44	0.95	7.02	3.98	212.2	216.1	186.0
8	190.4	246.1	1.28	0.86	5.90	3.50	210.0	213.7	187.4
9	193.9	248.0	1.33	0.90	6.50	3.65	211.6	217.8	190.1
					Av. wt. of paracetamol found per tablet			504.1 mg	446.3 mg
					Av. % labelled strength			100.8	89.3
					Coeff. of variation			2.47%	5.21%
					Error			0.82%	10.74%

+ based on the labelled strength.

Table 3:9. Results obtained for paracetamol assay by the official[153] method

Exp.No	Wt. of powdered tablets taken (mg)	Actual Wt. of paracetamol (mg)	Absorbance at 257 nm	Wt. of paracetamol found (mg)	% labelled strength
1	175.8	150.0	0.492	151.8	101.2
2	178.2	152.0	0.496	153.2	100.8
3	174.5	148.9	0.492	151.8	102.0
4	176.6	150.7	0.493	152.2	101.0
			Av. wt. of paracetamol found per tablet:		506.2 mg
			Av. % labelled strength:		101.2
			Coeff. variation:		0.67.

method the average weight of paracetamol obtained per tablet was 44.6 mg, with a high coefficient of variation (5.2%). Integration is therefore the method of choice for the parcetamol determination.

The average time taken for a single determination (including measurement of integrals and calculation) was about 10 mins after sample preparation.

3.3.2 'BRUFEN' TABLETS AND SUSPENSION

'Brufen' tablets (400 mg) and suspension (100 mg/5ml) have been assayed successfully by the use of nmr and employing 3-acetobenzythiophene as an internal standard. The results are given in tables 3:10 and 3:11, pages 222 and 223).

3-Acetobenzthiophene was used to demonstrate the use of an internal standard whose analytical resonance peak inteferes with that of the sample. The $C(CH_3)_2$ resonance of ibuprofen was used as the sample analytical peak. Since the standard peak came close to the CH_2 peak of ibuprofen (figure 3:2, page 221) it was necessary to integrate both and to correct for the contribution of the standard. Thus, the integral for the standard peak (I_s) was given by

$$I_s = A - \frac{B}{3}$$

where 'A' is the total integral for the standard and the CH_2 peaks and 'B' is the integral for the $C(CH_3)_2$ peaks.

The 'Brufen' tablets and suspension were also assayed by a

FIG 3:2 Assay of 'Brufen' tablets

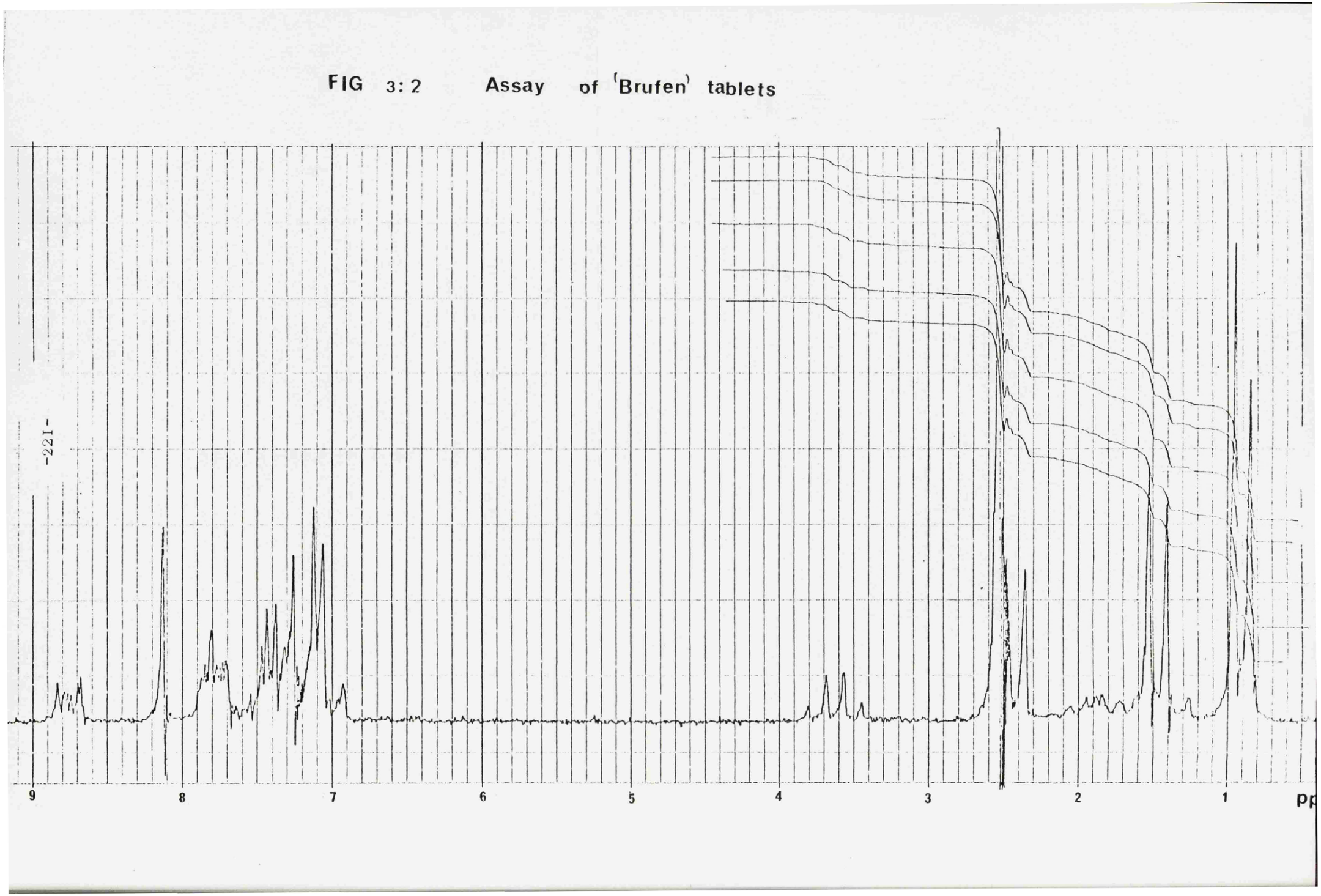


Table 3:10 NMR assay of 'Brufen' tablets

Av. Wt. of a tablet = 1.0309 g

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered sample (mg)	Av. integrals (cm) I_S	I_T	+Actual wt. of ibuprofen (mg)	Wt. of ibuprofen found (mg)	% labelled strength
1	48.0	134.7	2.55	5.01	52.3	55.2	105.6
2	66.9	136.2	2.60	3.14	52.9	47.3	89.4
3	70.3	136.0	2.65	3.50	52.8	54.4	103.1
4	50.2	129.2	2.58	3.95	50.1	45.0	89.8
5	56.9	134.3	2.59	4.45	52.1	57.2	109.8
6	63.6	135.8	2.59	3.59	52.7	51.6	98.0
7	56.5	132.9	2.57	3.75	51.6	48.3	93.7
						MEAN	99.7
						Coeff. Variation	7.89%

+ Based on the labelled strength

Table 3:11 NMR assay of 'Brufen' suspension (100 mg/5 ml)

Wt. per ml. of suspension = 1.0426 g

Expt. No.	Wt. of internal standard (mg)	Wt. of suspension (g)	Av. integrals (cm) I_S	Av. integrals (cm) I_T	*Actual wt. of ibuprofen (mg)	Wt of ibuprofen found (mg)	% labelled strength
1	40.2	2.1251	4.25	7.53	40.8	41.7	102.2
2	40.0	2.1034	4.18	7.27	40.4	40.7	100.9
3	40.9	2.1540	4.20	7.21	41.3	41.1	99.4
4	41.3	2.2500	4.30	7.61	43.2	42.8	99.1
5	40.8	2.1753	4.19	7.55	41.7	43.0	103.2
						MEAN	101.0%
						Coeff. variation	1.75%

* Based on the labelled strength.

Table 3:12 UV assay of 'Brufen' tablets

Absorbance of standard ibuprofen (0.00103%) at 222 nm = 0.430

Expt. No.	Wt. of powdered tablets (mg)	+Actual wt. of ibuprofen (mg)	Absorbance at 222 nm	Wt. of ibuprofen found (mg)	% labelled strength
1	70.4	27.3	0.576	27.2	99.6
2	69.6	27.0	0.561	26.5	98.2
3	70.3	27.3	0.574	27.1	99.2
4	70.1	27.2	0.570	26.9	98.9
				MEAN:	99.0%
				Coeff. variation	0.76%

+ Based on the labelled strength

Table 3:13 UV assay of 'Brufen' suspension

Expt. No.	Wt. of 'Brufen' suspension (mg)	+Actual wt. of ibuprofen (mg)	Absorbance at 222 nm	Wt. of ibuprofen found (mg)	% Labelled strength
1	200.6	19.2	0.413	19.5	101.5
2	208.5	20.0	0.424	20.1	100.2
3	202.1	19.4	0.413	19.5	100.6
4	216.3	20.7	0.436	20.6	99.8
				MEAN	100.5%
				Coeff. variation	0.83%

+Based on the labelled strength

UV method (page 285) for comparison with the nmr method. The results are given in tables 3:12 and 3:13, pages 224 and 225.

The results for both the suspension and tablet nmr assays compare favourably with those obtained by the UV method. However, a higher coefficient of variation was obtained for the nmr method.

3.3.3 EPHEDRINE HYDROCHLORIDE POWDER

The possibility of using nmr for the assay of Ephedrine HCl was investigated. With D_2O as the solvent, maleic acid as the internal standard and the aromatic resonance as the sample analytical peak, a very precise, accurate and fast analysis was obtained. The results are given in table 3:14. The method was compared with the official assay method[153]. It was found that the nmr method was slightly inferior to the official method (non-aqueous titration) with regard to accuracy and precision. The standard deviation for the nmr method for eleven results was 1.04 whereas the standard deviation for the non-aqueous titration method for only four results was 0.03. However, the nmr method was faster than the non-aqueous titration. Another advantage of the nmr method was that it required less material for the analysis than the titration. The results for the non-aqueous titration are given in table 3:15, page 228.

3.3.4 PSEUDO-EPHEDRINE HYDROCHLORIDE POWDER

As with ephedrine hydrochloride, pseudo-ephedrine hydrochloride gives a singlet for the aromatic protons in D_2O. Using maleic acid as the internal standard, results similar but of higher standard deviation, to that of ephedrine hydrochloride were obtained. The results are given in table 3:16, page 229.

Table 3:14 NMR assay of ephedrine HCl

Expt. No.	Wt. of internal standard (mg)	Actual wt. of ephedrine HCl (A; mg)	Av. integrals (cm) I_s	I_T	Wt. of ephedrine HCl obtained (B,mg)	% w/w (B/Axl00)
1	40.1	37.5	4.03	5.43	37.7	100.5
2	41.3	43.3	4.07	5.25	44.1	101.8
3	41.9	55.1	4.08	7.73	55.2	100.2
4	45.6	58.0	4.12	7.55	58.1	100.2
5	47.9	60.0	4.14	7.50	60.3	100.4
6	42.5	53.4	4.09	7.46	53.6	100.4
7	40.3	40.2	4.04	6.00	41.6	103.5
8	35.6	35.5	4.01	5.79	35.7	100.6
9	42.7	50.1	4.10	7.03	50.9	101.5
10	44.4	43.0	4.12	5.79	43.4	100.9
11	46.5	46.3	4.11	5.89	46.3	100.0
					MEAN	100.9%
					Coeff. variation	1.03%

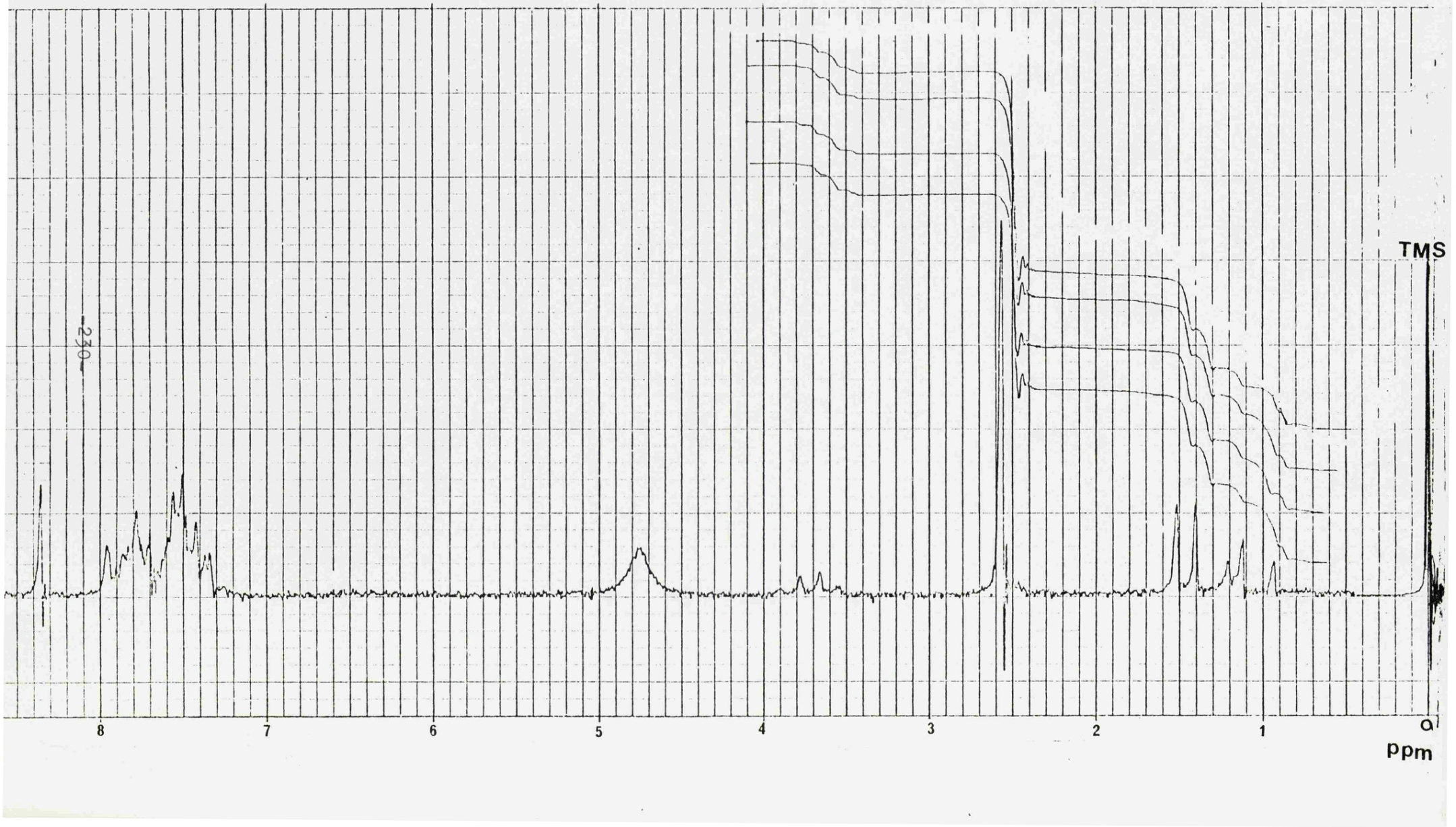
Table 3:15 Non-aqueous titration assay method[153] for ephedrine HCl

Expt. No.	Actual wt. of ephedrine HCl (A; mg)	Vol. of 0.1N perchloric acid (ml)	Wt. of ephedrine HCl obtained (B; mg)	% w/w (B/A x 100)
1	172.5	8.55	172.4	100.0
2	170.4	8.45	170.4	100.0
3	174.4	8.65	174.5	100.0
4	170.4	8.45	170.4	100.0
			MEAN	100.0%
			Coeff. variation	0.03%

Table 3:16 NMR assay of pseueo-ephedrine HCl

Expt. No.	Wt. of internal standard (mg)	Actual wt of pseudo-ephedrine HCl taken (A; mg)	Av.integrals (cm) I_s	I_T	Wt. of pseudo-ephedrine HCl obtained (B; mg)	% w/w (B/Axl00)
1	35.6	33.2	4.00	5.43	33.6	101.2
2	37.2	38.9	4.23	6.18	37.8	97.2
3	46.3	70.2	4.03	8.65	69.1	98.4
4	44.0	62.1	4.65	9.46	62.2	100.2
5	40.1	53.4	4.01	7.83	54.4	101.9
6	49.2	65.9	4.05	8.21	69.4	105.3
7	38.5	40.5	4.48	6.83	40.6	100.2
8	35.2	45.0	4.00	7.34	44.9	99.8
9	38.7	56.1	4.01	8.35	56.0	99.8
					MEAN	100.4%
					Coeff. variation	2.28%

FIG 3:3 Assay of 'Orudis'



3.3.5 'ORUDIS CAPSULES'

Orudis contains ketoprofen, 50 mg per capsule. The ketoprofen content of "Orudis" has been determined using 3-acetobenzthiophene as the internal standard. The C-CH_3 signal of ketoprofen was used as the sample analytical peak. The results are given in table 3:17, page 232.

Some resonance peaks due to the excipients appeared in the spectrum close to the sample analytical peak (figure 3:3, page 230). However they did not interfere with the analysis. This is clear from the results obtained which compare favourably with the results obtained by a UV method (shown in table 3:18, page 233).

3.3.6 'INDERAL'

Inderal is a tablet preparation containing propranolol hydrochloride (80 mg per tablet) as the active ingredient.

The propranolol hydrochloride content of 'Inderal' tablets has been determined successfully by nmr using pure propranolol hydrochloride as internal standard. The experiment illustrates the fact that an internal standard based on the pure form of the test sample can be as good as the other internal standards. Two determinations were carried out at a time. In one case the nmr spectrum of the sample alone was recorded, in the other case both the sample and the pure propranolol hydrochloride were dissolved in the same quantity of solvent and the spectrum recorded. The aromatic peaks were used as the analytical peaks. The results are given in table 3:19, page 236.

Table 3:17 NMR assay of 'Orudis' capsules

Av. wt. of a capsule (including shell) = 0.2032 g

Av. wt. of the content of a capsule = 0.1385 g

Expt. No.	Wt. of internal standard (mg)	Wt. of powder taken (mg)	Av. integrals (cm) I_s	I_T	Actual wt. of ketoprofen (mg)	Wt. of ketoprofen found (mg)	% labelled strength
1	68.1	138.5	5.35	2.72	50.0	48.6	97.1
2	68.5	140.2	5.40	2.76	50.6	49.7	98.2
3	68.9	139.5	5.41	2.74	50.4	49.0	97.2
4	67.8	138.3	5.02	2.56	49.9	48.4	96.9
5	68.3	140.1	5.36	2.75	50.6	49.7	98.3
6	68.6	138.6	5.42	2.74	50.0	49.5	99.0
7	68.1	138.5	5.34	2.72	50.0	46.9	93.8
8	67.9	139.1	5.29	2.71	50.2	49.1	97.8
9	67.9	137.9	5.32	2.70	49.8	48.2	96.8
10	68.3	140.0	5.22	2.67	50.5	48.4	95.8
						MEAN	97.1%
						Coeff. variation	1.65%

Table 3:18 UV assay of 'Orudis' capsules

Absorbance of standard ketoprofen (0.00131. w/v) at 253 nm = 0.89

Exp. No.	Wt. of powder taken (mg)	Actual wt. of ketoprofen (mg)	Absorbance at 253 nm	Wt. of ketoprofen found (mg)	% labelled strength
1	138.5	50.0	0.82	48.3	96.6
2	"	"	0.84	49.4	98.8
3	"	"	0.82	48.3	96.6
4	"	"	0.83	48.9	97.8
				MEAN	97.6%
				Coeff. variation	1.07%

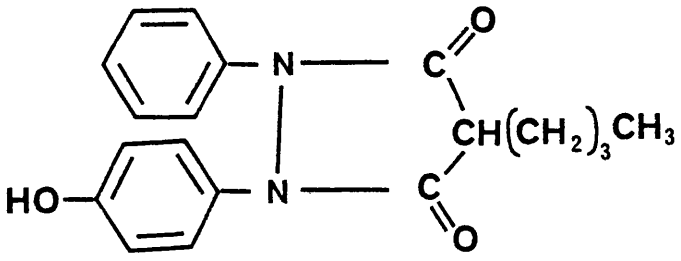
The results were compared with those obtained by the official[153] method (table 3:20, page 237). The official method is a UV method of assay which involves extraction of the propranolol hydrochloride into methanol and measurement of the extinction at 290 nm, $E^{1\%}_{1cm}$ = 210 at 290 nm .

The nmr and official methods gave the average labelled strength as 96.4% and 98.8% respectively. Though the accuracy of the two methods compares favourably, the official method is more precise than the nmr method.

The $C(CH_3)_3$ signal could not be used for the analysis because of signal overlap with excipients.

3.3.7 'TANDERIL' (Geigy)

'Tanderil' is a tablet preparation containing oxyphenbutazone (3:2) (100 mg per tablet).



(3:2)

The nmr assay of tanderil involves the use of 3-acetobenzthiophene and the $(CH_2)_3$-CH_3 resonance signals at δ0.8 to 2.2 ppm (figure 3:4, page 235). The results are given in table 3:21, page 239.

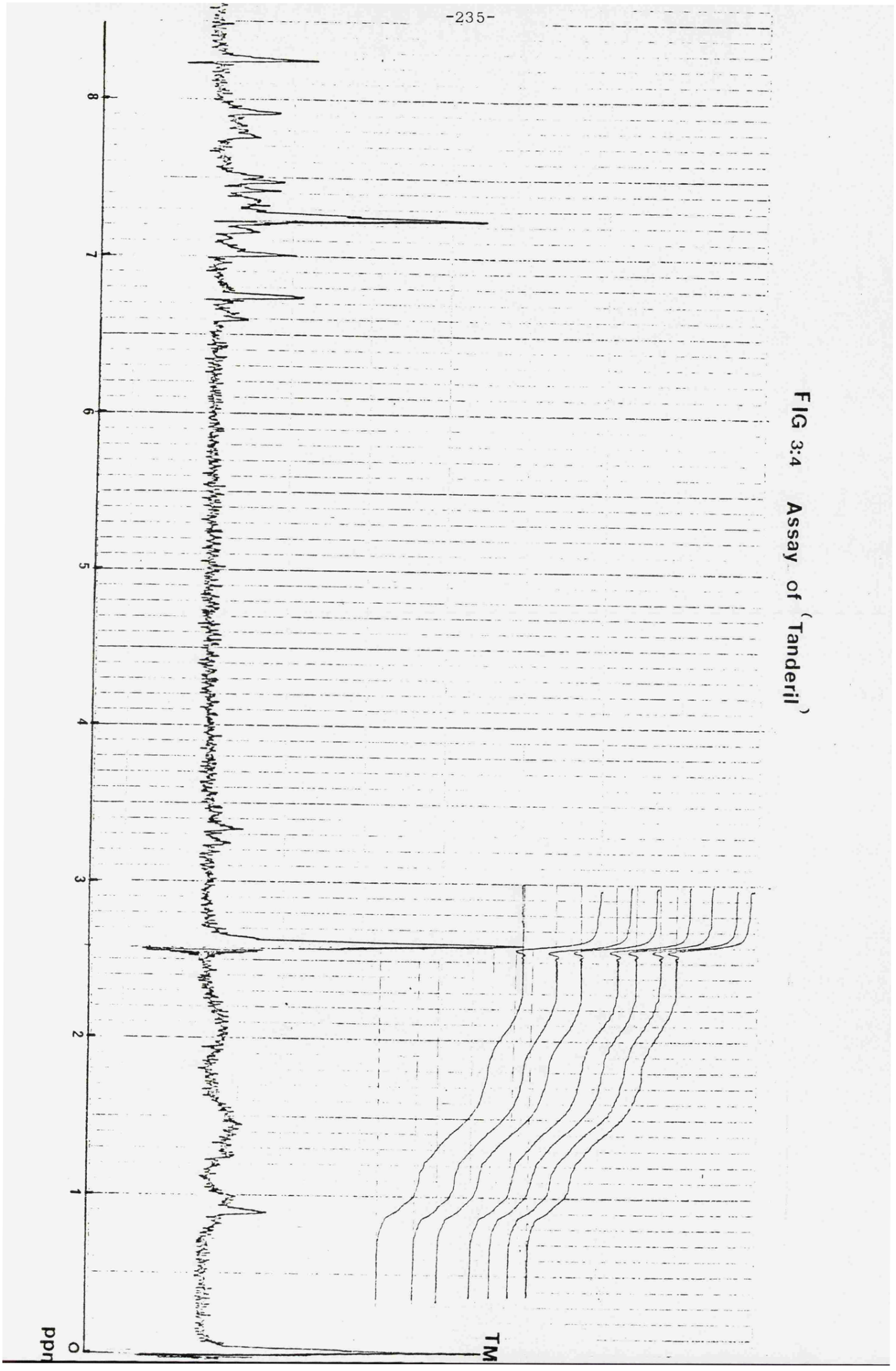


FIG 3:4 Assay of 'Tanderil'

Table 3:19 Results for the nmr assay of 'Inderal' tablets

Av. wt. of a tablet = 286.3 mg

Exp. No.	Wt. of internal standard (mg)	*Wt. of powdered tablets taken (mg)	Av. integrals (cm) I_s+I_T	I_T	I_s	+Actual wt. of propranolol HCl (mg)	Wt. of propranolol HCl found (mg)	% labelled strength
1	68.8	176.1	5.17	2.10	3.07	49.2	47.1	95.8
2	69.0	172.1	5.39	2.19	3.20	48.1	47.2	98.1
3	68.5	176.1	5.13	2.08	3.05	49.2	46.7	94.9
4	68.3	166.8	5.32	2.12	3.10	46.6	46.7	100.1
5	69.1	166.0	5.01	2.00	3.01	46.4	45.9	99.0
6	68.9	173.2	5.05	2.03	3.02	48.4	46.3	95.6
7	67.9	175.7	5.27	2.16	3.11	49.1	47.2	96.1
8	68.2	170.7	5.30	2.11	3.19	47.7	45.1	94.6
9	68.4	171.4	5.18	2.09	3.09	47.9	46.3	96.6
10	69.3	182.9	5.28	2.15	3.13	51.1	47.6	93.1
						Av. wt. of propranolol HCl found per tablet		77.1 mg
						Av. % labelled strength		96.4
						Coeff. variation		2.21.

+ Based on the labelled strength

*The same weight was used as the test sample in the same vol. of solvent (1.0 ml).

Table 3:20 Results for the 'Inderal' assay by the official[153] method.

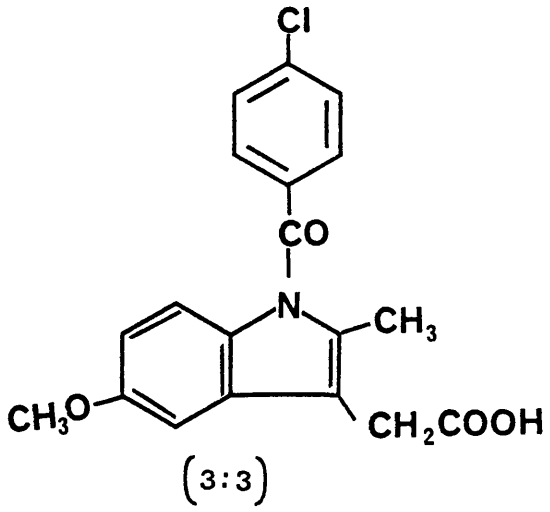
Expt. No.	Wt. of powdered tablets taken (mg)	+Actual wt. of propranolol HCl	Absorbance at 290 nm	Wt. of propranolol HCl found (mg)	% labelled strength
1	71.6	20.0	0.414	19.7	98.5
2	76.9	21.5	0.447	21.3	99.2
3	72.3	20.2	0.420	20.0	98.9
4	73.7	20.6	0.426	20.3	98.8
				Av. % labelled strength	98.8
				Coeff. variation	0.52.

+ Based on the labelled strength

The official assay method[153] for oxyphenbutazone tablets utilises an acid-base titration method. The results obtained using this method are given in table 3:22, page 240. An average of 97.3% of the lablled strength and a variation of 1.32% were obtained by the nmr method. The official method had 98.5% as the % labelled strength with 0.72% variation. With regard to accuracy, the methods are equally good. In terms of sample size, the nmr has the advantage over the official method. The official method requires about 500 mg of oxyphenbutazone for the analysis, whereas the nmr method requires less than 90 mg. The nmr method is more rapid, though less precise, than the official method.

3.3.8 'INDOCID' CAPSULES (M.S.D.)

'Indocid' capsules contain indomethacin (3:3) (25 mg per capsule).



(3:3)

The nmr assay of 'Indocid' involves the use of vanillin as an internal standard (see table 3:1, page 201) and the C-CH_3 signal of the indomethacin at δ2.38 ppm as the sample analytical peak. The results are given in table 3:23, page 242.

Table 3:21 NMR assay of 'Tanderil' tablets

Av. wt. of a tablet = 186. 3 mg

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered tablets (mg)	Av.integrals (cm) I_S	I_T	+Actual wt. of oxyphenbutazone (mg)	Wt.of oxyphenbutazone found (mg)	% labelled strength
1	58.2	164.3	4.00	9.5	88.2	85.1	96.6
2	60.5	162.2	4.05	9.0	87.0	82.2	94.5
3	59.9	148.2	4.08	8.7	79.5	77.9	98.0
4	61.1	146.2	4.12	8.4	78.4	76.7	97.8
5	58.7	150.0	4.04	8.8	80.5	78.2	97.2
6	59.1	149.2	4.06	8.7	80.1	79.5	99.3
7	58.5	148.9	4 .05	8.7	79.9	77.0	96.3
8	60.7	151.1	4.10	8.7	81.1	78.9	97.3
9	59.7	150.2	4.09	8.8	80.6	78.6	97.5
10	60.2	149.5	4.03	8.6	80.2	78.7	98.1
						MEAN	97.3%
						Coeff. variation	1.32%

+ Based on the labelled strength

Table 3:22 Results for the 'Tanderil' assay by the official[153] method

Exp. No.	Wt. of powdered tablets (g)	Vol. (ml) of 0.1N (0.995) NaOH required by the oxyphenbutazone	+Actual wt. of oxyphenbutazone (mg)	Wt. of oxyphenbutazone found (mg)	% labelled strength
1	1.210	15.85	520.0	511.7	98.4
2	1.167	15.33	501.2	494.7	98.7
3	1.186	15.64	509.5	504.9	99.1
4	1.163	15.15	499.6	489.1	97.9
				MEAN	98.5
				Coeff. variation	0.72%

+Based on the labelled strength

A UV method is the official assay method[153] for indomethacin capsules. This involves the measurement of the extinction of the sample in methanol (buffered with a solution of standard pH 7.2) at 318 nm. $E^{1\%}_{1cm}$ = 193 at 318 nm . The results obtained for this method are given in table 3:24, page 243 .

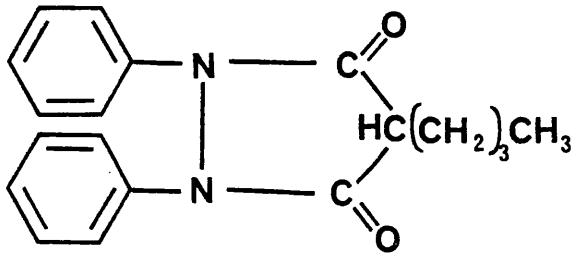
The nmr method gave a higher % labelled strength (104.6%) than the official method (100.9.). Moreover, the nmr method was less precise (coeff. var. 2.64%) than the official method (coeff. var. 0.72%).

3.3.9 'BUTAZOLIDINE ALKA'

Butazolidine Alka is a tablet preparation containing the following active ingredients:

Dried alum. hydrox. gel	100 mg
Magnesium trisilicate	150 mg
Phenylbutazone	100 mg

The phenylbutazone (3:4) content has been determined using nmr and



(3:4)

3-acetobenzthiophene as internal standard. The signals from the nine protons of the alkyl chain ($CH_2)_3CH_3$ were used as the analytical

Table 3:23 NMR assay of 'Indocid' capsules (25 mg)

Av. wt. of the content of a capsule = 262.2 mg

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered sample (mg)	Av. integrals (cm) I_s	I_T	+Actual wt. of indomethacin (mg)	Wt. of Indomethacin found (mg)	% labelled strength
1	60.2	290.4	3.65	2.31	27.7	29.9	108.1
2	65.1	240.0	3.92	1.82	22.9	23.7	103.7
3	68.0	260.0	4.02	1.96	24.8	26.0	105.2
4	62.5	259.2	3.74	1.99	24.7	26.0	105.4
5	68.4	262.2	4.11	2.02	25.0	26.3	105.2
6	68.7	242.6	4.18	1.84	23.1	23.7	102.6
7	60.9	240.2	3.69	1.78	22.9	23.0	100.6
8	63.6	244.5	3.78	1.78	23.3	23.4	100.6
9	64.9	259.5	3.82	1.95	24.7	26.0	105.3
10	68.1	249.9	4.09	1.99	23.8	25.9	108.8
						MEAN	104.6%
						Coeff. variation	2.64%

+Based on the labelled strength

Table 3.24 Assay of Indomethacin capsules by the official[153] method

Expt. No.	Wt. of powdered sample (mg)	+Actual wt. of indomethacin (mg)	Absorbance at 318 nm	Wt. of indomethacin found (mg)	% labelled strength
1	522.3	49.8	0.482	49.9	100.2
2	524.4	50.0	0.486	50.4	100.8
3	529.6	50.5	0.494	51.2	101.3
4	520.2	49.6	0.483	50.1	101.1
				MEAN	100.9%
				Coeff. variation	0.72%

+Based on the labelled strength

Table 3:25 Assay of 'Butazolidine Alka' by nmr method

Av. wt. of a tablet = 539.5 mg

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered sample (mg)	Av. integral (cm) I_s	I_T	+Actual wt. of phenylbutazone (mg)	Wt. of phenyl-butazone found (mg)	% labelled strength
1	57.5	203.5	3.95	4.59	37.7	38.7	102.5
2	57.9	202.2	4.01	4.58	37.5	38.5	102.7
3	57.1	200.5	3.91	4.47	37.2	38.0	102.2
4	58.2	210.0	4.05	4.66	38.9	39.0	100.2
5	56.9	204.2	3.82	4.49	37.8	39.0	103.0
6	59.2	200.0	4.11	4.54	37.1	38.1	102.9
7	58.1	208.1	4.0	4.65	38.6	39.4	102.3
8	56.9	210.2	3.81	4.6	39.0	40.0	102.6
9	57.6	204.4	3.89	4.51	37.9	38.9	102.7
10	58.0	203.0	4.0	4.56	37.6	38.5	102.3
						MEAN	102.3%
						Coeff. variation	0.78%

+ Based on the label strength

Table 3:26 Results for the 'Butazolidine Alka' assay by the official[153] method

Expt. No.	Wt. of powdered sample (g)	Vol.(ml) of 0.1N (0.995) NaOH required by the phenylbutazone	+Actual wt. of phenylbutazone (mg)	Wt. of phenyl-butazone found (mg)	% labelled strength
1	2.7844	17.02	516.1	522.3	101.2
2	2.7185	16.57	503.9	508.4	100.9
3	2.7520	16.71	510.1	512.7	100.5
4	2.7466	16.69	509.1	512.2	100.6
				MEAN	100.8%
				Coeff. variation	0.57%

+Based on the labelled strength

peak (see table 3:33, page 254). The results are given in table 3:25, page 244.

The U.S.P.[171] employs a UV method for assay of phenylbutazone tablets by measuring the extinction of the sample in O.1N NaOH at 264 nm. The B.P.[153] method, however, uses an acid-base titration method. The B.P. method was followed for comparison with the nmr method. The results are given in table 3:26 (page 245).

The results for the two methods compare favourably with each other, both in accuracy and precision. However, the nmr method has the advantage of being more rapid and also requiring less material for the analysis.

3.3.10 'NAPROSYN'

Naprosyn is a tablet preparation containing Naproxen 250mg.

With 3-acetobenzthiophene as the internal standard and the OCH_3 and *CH* signals of naproxen as the sample analytical peaks, the nmr assay of 'Naprosyn' gave an average of 95.4% labelled strength with a variation of 1.91%. The results are given in table 3:27, page 247. The average % labelled strength obtained by a UV method (page 285) for 'Naprosyn' was 100.2% with a variation of 0.51%. The results for the UV method are given in table 3:28 (page 248).

3.3.11 'BENORAL' TABLETS AND SUSPENSION

'Benoral' (Winthrop) preparations contain benorylate (2:33) (see section 4.1, page 289). Each tablet contains 750 mg benorylate, and

Table 3:27 NMR assay of 'Naprosyn' tablets

Av. wt. of a tablet = 380.9 mg

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered tablets (mg)	Av. integral (cm) I_s	Av. integral (cm) I_T	+Actual wt. of Naproxen (mg)	Wt. of Naproxen found (mg)	% labelled strength
1	91.8	187.6	3.52	4.81	123.1	122.9	99.8
2	90.9	185.2	3.49	4.72	121.5	120.6	99.3
3	91.6	180.0	3.50	4.45	118.1	114.1	96.6
4	92.0	184.6	3.61	4.77	121.1	119.2	98.4
5	90.5	182.2	3.40	4.36	119.5	113.9	95.3
6	90.7	184.9	3.42	4.50	121.3	116.9	96.4
7	90.5	183.6	3.41	4.45	120.4	115.9	96.3
8	91.2	186.2	3.49	4.71	122.2	120.7	98.8
9	92.3	187.2	3.65	4.70	122.8	116.6	95.0
10	92.1	187.9	3.60	4.80	123.3	120.3	97.6
						MEAN	97.4%
						Coeff. variation	1.91%

+Based on the labelled strength

Table 3:28 UV assay of Naprósyn tablets.

Absorbance of standard Naproxen (0.00205% w/v) in MeOH at 273nm = 0.532

Expt. No.	Wt. of powdered sample (mg)	+Actual wt. of Naproxen (mg)	Absorbance at 273 nm	Wt. of Naproxen found (mg)	% labelled strength
1	30.5	20.0	0.522	20.1	100.5
2	39.8	19.6	0.509	19.6	100.1
3	30.7	20.1	0.522	20.1	99.9
4	31.0	20.3	0.529	20.4	100.3
				MEAN	100.2%
				Coeff. variation	0.51%

+Based on labelled strength

the suspension contains 4 g benorylate in 10 ml.

'Benoral' tablets and suspension have been assayed using nmr and employing maleic acid as the internal standard. The signals for the two acetyl groups (table 3:33, page 254) were used for the analysis. The results are given in tables 3:29, page 250 and 3:30, page 251.

UV assay procedures for the tablets (see section 3.6.2.8, page 286) and suspension (see section 3.6.2.9, page 286) were also carried out for comparison with the nmr method. The results are given in tables 3:31 and 3:32, pages 252 and 253.

Both the nmr and UV assay results for the 'Benoral' tablets were close. However, the coefficient of variation was higher for the nmr method (4.78%) than for the UV method (0.77%).

The nmr method for 'Benoral' suspension gave very high results compared with those of the UV method and the labelled strength. This might have been due to an irrelevant peak coming under the sample analytical peaks. The method is therefore not suitable for the 'Benoral' suspension.

Table 3:29 NMR assay of 'Benoral' tablets

Av. wt. of a tablet = 0.7562 g

Expt. No.	Wt. of internal Standard (mg)	Wt. of powdered tablets (mg)	Av. integrals (cm) I_s	Av. integrals (cm) I_T	+Actual wt. of benorylate (mg)	Wt. of benorylate obtained (mg)	% labelled strength
1	90.1	120.1	2.95	4.51	119.1	124.0	104.1
2	120.3	114.7	3.20	3.57	113.8	120.7	106.1
3	115.2	129.4	3.15	3.81	128.3	125.2	97.6
4	95.6	111.2	2.99	3.57	110.3	102.5	92.9
5	100.2	104.5	3.02	3.78	103.6	112.8	108.9
6	96.3	129.2	3.00	4.12	128.1	118.9	98.2
7	105.7	116.9	3.11	4.01	115.9	122.5	105.7
8	120.1	117.7	3.20	3.52	116.7	119.0	102.0
9	90.8	110.0	2.96	3.93	109.1	108.6	99.5
10	100.5	114.1	3.04	3.77	113.2	112.0	98.9
						MEAN	101.4%
						Coeff. variation	4.78%

+Based on labelled strength

Table 3:30 NMR assay of 'Benoral' suspension

Wt. per ml. of suspension = 1.2235 g

Expt. No.	Wt. of internal standard (mg)	Wt. of suspension (mg)	Av. integrals (cm) I_S	I_T	+Supposed wt.of benorylate (mg)	Wt. of benoryl-ate obtained (mg)	% labelled strength
1	120.0	904.2	3.25	10.51	295.7	349.2	118.1
2	129.2	873.2	3.38	8.66	286.3	297.8	104.0
3	126.3	873.2	3.32	9.80	286.3	335.5	117.2
4	123.8	972.4	3.30	9.65	318.8	325.5	102.1
5	120.3	829.3	3.27	9.58	271.1	316.9	116.9
6	129.5	829.3	3.40	8.99	271.1	308.0	113.6
7	130.1	819.7	3.43	8.91	268.0	303.9	113.4
8	128.3	819.9	3.36	8.55	268.1	293.8	109.6
9	129.6	826.8	3.41	8.11	270.3	277.1	102.5
10	130.5	842.0	3.44	8.62	275.3	294.0	106.8
						MEAN	110.4%
						Coeff. variation	5.67%

+Based on the labelled strength

Table 3:31 UV assay of 'Benoral' tablets

Absorbance of standard benorylate (5.175×10^{-4}% w/v) in MeOH at 240 nm = 0.410

Expt. No.	Wt. of powdered tablets (mg)	Actual wt. of benorylate (mg)	Absorbance at 240 nm	Wt. of benorylate obtained (mg)	% labelled strength
1	22.6	22.4	0.444	22.4	100.0
2	23.3	23.1	0.457	23.3	100.8
3	22.0	21.8	0.432	21.8	100.0
4	22.9	22.7	0.450	22.9	101.0
				MEAN	100.5%
				Coeff. variation	0.77%

Table 3:32 UV assay of 'Benoral' suspension

Expt. No.	Wt. of suspension taken (mg)	Actual wt. of benorylate (mg)	Absorbance at 240nm	Wt. of benorylate obtained (mg)	% labelled strength
1	61.2	20.0	0.394	19.9	99.5
2	65.7	21.5	0.422	21.3	98.9
3	60.5	19.8	0.392	19.8	100.0
4	63.0	20.6	0.406	20.5	99.8
				MEAN	99.6%
				Coeff. variation	0.73%

Table 3:33 Summary of the conditions for the assay of the preparations in section 3.3.

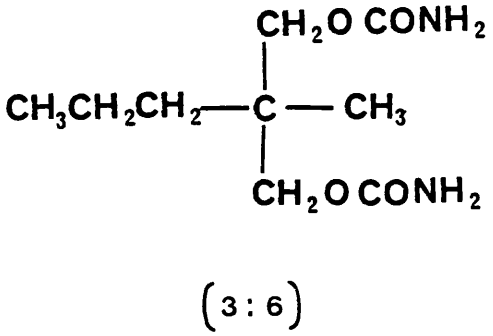
Preparation	Active ingredient	Equivalent Wt. (E_T)	Sample analytical group	Resonance position (ppm)	Internal standard	Solvent (Vol;ml)
Panadol	Paracetamol	50.4	$NCOCH_3$	2.01	3,4 dimethoxy-benzoic acid	$CDCl_3$/DMSO(d_6) (1:3) (1.2)
Brufen tab.						(1)
Brufen suspension	ibuprofen	34.4	$CH(CH_3)_2$	0.5-4.0	3-acetobenzthio-phene	CCl_4 (2)
Ephedrine HCl Pseudo-ephedrine HCl	-	40.3	aromatic peaks	aromatic position	Maleic acid	D_2O (0.5)
Butazolidine Alka	Phenylbutazone	34.2	$(CH_2)_3-CH_3$	0.8-2.4	3-acetobenz-thiophene	$CDCl_3$ (0.7)
Orudis capsules	ketoprofen	84.8	$C-CH_3$	1.4-1.6	3-acetobenzthio-phene	$CDCl_3$ (1)
Inderal	propranolol HCl	-	aromatic peaks	aromatic position	pure propranolol HCl	$CDCl_3$/DMSO(d_6) (3:1) (1)
Tanderil	oxyphenbutazone	36.0	$(CH_2)_3-CH_3$	0.8-2.2	3-acetobenzthio-phene	$CDCl_3$ (1)
Indocid capsules	indomethacin	119.3	$C-CH_3$	2.38	vanillin	$CDCl_3$ (0.5)
Naprosyn	Naproxen	57.6	OCH_3 & CH	3.5-4.15	3-acetobenzthio-phene	$CDCl_3$ (2)
Benoral tab. Benoràl sus.	Benorylate	52.2	$COCH_3$ & $NCOCH_3$	2-2.3	maleic acid	(2) DMSO(d_6)/ $CDCl_3$ (3:1) (3)

3.4 ASSAY OF FORMULATIONS CONTAINING MORE THAN ONE ACTIVE INGREDIENT

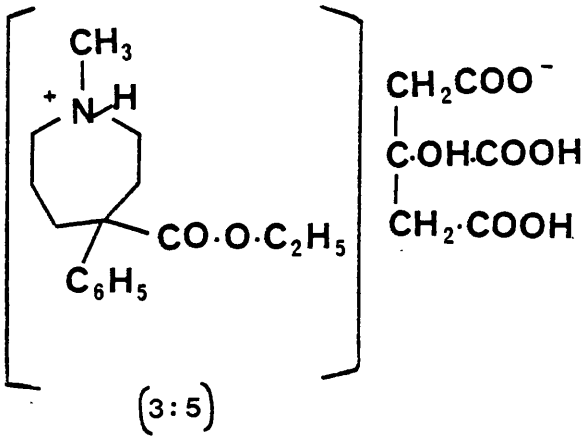
3.4.1 'EQUAGESIC' TABLETS

'Equagesic' tablets contain the following active ingredients per tablet:

Ethoheptazine citrate	75 mg
Meprobamate	150 mg
Aspirin	250 mg
$CaCO_3$	75 mg



(3:6)



(3:5)

The Ethopheptazine citrate (3:5), Meprobamate (3:6) and Aspirin content of 'Equagesic' tablets have been determined successfully utilising their combined solubilities in acetone (d_6) and DMSO(d_6), and using pure meprobamate as the internal standard for the meprobamate and aspirin content.

The determination of the ethoheptazine citrate, meprobamate and aspirin content together by the use of nmr is not feasible due to overlap of resonance peaks. Meprobamate and aspirin are readily soluble in acetone (d_6), whereas ethoheptazine citrate is insoluble.

Extraction of the meprobamate and aspirin with acetone (d_6) leaves ethoheptazine citrate, $CaCO_3$ and other excipients behind. When the residue is treated with DMSO(d_6), the ethohephazine citrate is dissolved. Maleic acid was used as an internal standard for the ethoheptazine citrate. For the meprobamate and aspirin, the 2(CH_2O) signal at δ3.8 ppm belonging to the meprobamate and the $COCH_3$ signal at δ2.2 ppm for the aspirin were employed for the analysis. Two simultaneous equations were used for the calculations of the aspirin and meprobamate contents. The method of calculation is given below and the experimental details are given in section 3.6.3.1, page 286.

Method of calculation

Meprobamate and aspirin determination

Explanation of symbols used:

Int. A - Integral of aspirin $COCH_3$ signal in the 1st sample.

Int. A' - Integral of aspirin $COCH_3$ in the 2nd sample.

Int. B - Integral for the 2(CH_2O) signal of meprobamate in the 1st sample

Int. Bs - Total integral for the standard meprobamate and the meprobamate content of the tablets in the 2nd sample.

E_A - Equivalent wt. of aspirin = 60.05

E_B - Equivalent wt. of meprobamate = 54.6

W_A - Wt. of aspirin in the sample.

W_B - Wt. of meprobamate in the sample.

W_{BS} - Wt. of standard meprobamate added to the sample.

$$\text{Ratio} \quad \frac{\text{Int. A}}{\text{Int. B}} = \frac{E_B \times W_A}{E_A \times W_B} \qquad \ldots(1)$$

Similarly $$\frac{\text{Int. A'}}{\text{Int. Bs}} = \frac{E_B \times W_A}{E_A(W_B+W_{Bs})} \quad \ldots(2)$$

From equation (1)

$$W_A = \frac{\text{Int. A} \times E_A \times W_B}{\text{Int. B} \times E_B}$$

$$= KW_B \quad \ldots(3)$$

where $K = \frac{\text{Int. A} \times E_A}{\text{Int. B} \times E_B}$

From equation (2) $$W_A = \frac{\text{Int. A'} \times E_A \times (W_B+W_{Bs})}{E_B \times \text{Int. (Bs)}}$$

$$= K'(W_B + W_{Bs})$$

$$= K'W_B + K'W_{BS}$$

$$= K'W_B + S \quad \ldots(4)$$

where $K' = \frac{\text{Int. A'x}E_A}{\text{Int.B}_s \text{x}E_B}$

and $S = K'W_{BS}$

$$\therefore \quad KW_B = K'W_B + S$$

$$KW_B - K'W_B = S$$

$$W_B(K - K') = S$$

$$\therefore \quad W_B = \frac{S}{K - K'} \quad \ldots(5)$$

W_A can be obtained by substituting W_B in either (4) or (3).

For the ethoheptazine citrate determination the aromatic signals were employed as the analytical peaks. The results for the aspirin and meprobamate are given in table 3:34, page 259 and those for ethoheptazine citrate are given in table 3:35, page 260.

The official assay methods for determining the aspirin[153] meprobamate[153] and ethoheptazine[172] contents were also performed. For the aspirin content, the method involves extraction procedures followed by conversion into salicylic acid. The latter is then treated with excess 0.1N bromine (50 ml) and back titrated with 0.1N sodium thiosulphate after addition of potassium iodide solution. The results are given in table 3:38, page 263. The results for the official method (av. labelled strength, 98.0%) compare well with those obtained by the nmr method (97.4%).

The official method for meprobamate content is based on conversion of the amide into ammonia. The ammonia liberated is distilled into 0.1N H_2SO_4 (50 ml) and the excess H_2SO_4 titrated with 0.1N NaOH. The results are given in table 3:36, page 261. The average % labelled strength obtained (101.0) agrees with that obtained by the nmr method (102.6%).

The N.F.[172] method was followed for the ethoheptazine citrate content. The method involves conversion of the salt (ethoheptazine citrate) to the base (ethoheptazine) and extraction of the latter into CCl_4. The base is determined by a non-aqueous titration involving the use of 0.05N perchloric acid in glacial acetic acid. The results (table 3:37, page 262) agree with those obtained by the nmr method.

Table 3:34 NMR assay of 'Equagesic' tablets - meprobamage and aspirin content

Av. wt. of a tablet = 667.2 mg.

Expt. No.	Wt. of standard meprobamate (W_{BS};mg)	Wt. of powdered sample (mg)	Av. integrals (cm) A	A'	B	BS	MEPROBAMATE Actual wt. of meprobamate (mg)	Wt. of meprobamate found (W_B;mg)	% labelled strength	ASPIRIN Actual wt. of aspirin (mg)	Wt. of aspirin found (W_A;mg)	% labelled strength
1	83.6	252.7	1.81	1.79	1.29	3.10	56.81	58.4	102.8	94.69	90.2	95.3
2	83.5	252.5	1.86	1.84	1.30	3.11	56.78	58.5	103.0	94.61	92.6	97.9
3	83.5	252.8	1.89	1.87	1.30	3.12	56.83	58.7	103.3	94.72	94.7	100.0
4	83.0	252.5	1.87	1.86	1.29	3.11	56.78	58.0	102.1	94.61	92.7	98.0
5	83.7	252.2	1.80	1.79	1.28	3.10	56.70	58.3	102.8	94.50	90.2	95.4
6	83.4	253.0	1.86	1.84	1.28	3.09	56.88	57.9	101.8	94.79	92.5	97.6
							MEAN		102.6%			97.4%
							Coeff. variation		0.80%			1.21%

Table 3:35 NMR assay of equagesic tablets - ethoheptazine citrate content

Expt. No.	Wt. of maleic acid (mg)	Wt. of powdered sample (mg)	Av.integrals (cm) I_s	I_T	Actual wt. of ethoheptazine citrate (mg)	Wt. of ethoheptazine citrate found (mg)	% labelled strength
1	40.2	252.7	6.30	2.79	28.41	27.8	97.8
2	40.9	252.5	6.35	2.70	28.38	27.2	95.8
3	40.3	252.8	6.31	2.84	28.42	28.3	99.5
4	39.8	252.5	6.28	2.78	28.38	27.5	97.0
5	40.4	252.2	6.34	2.64	28.35	26.3	92.9
6	39.8	253.0	6.30	2.73	28.44	27.0	95.1
						MEAN	96.4%
						Coeff. variation	2.38%

Table 3:36 Official assay method[153] for meprobamate in 'equagesic' tablets

Expt. No.	Powdered sample (g)	Vol. of 0.1N NaOH required to neutralise the excess 0.1N H_2SO_4 (B; ml)	Vol. of 0.1N H_2SO_4 reqd. by the meprobamate (50-B; ml)	+Actual wt. of meprobamate (mg)	Wt. of meprobamate FOUND (mg)	%labelled strength
1	1.3344	22.28	27.72	300.00	302.4	100.8
2	1.3298	22.26	27.74	298.97	302.6	101.2
3	1.3458	22.02	27.98	302.56	305.3	100.9
4	1.3344	22.20	27.80	300.00	303.3	101.1
					MEAN	101.0%
					Coeff. variation	0.40%

Table 3:37 Official assay method[172] for Ethoheptazine citrate in 'Equagesic' tablets

Expt. No.	Wt. of powdered sample (g)	Vol. (ml) of 0.025N perchloric acid required by the ethoheptazine	+Actual wt. of ethoheptazine citrate (mg)	Wt. of ethoheptazine found (mg)	% labelled strength
1	1.3400	13.09	150.63	148.4	98.5
2	1.3344	13.12	150.00	148.8	99.2
3	1.3299	13.06	149.49	148.1	99.1
4	1.3367	13.10	150.26	148.6	98.9
				MEAN	98.9%
				Coeff. variation	0.55%

+ Based on the labelled strength

Table 3:38 Official assay method[153] for the aspirin content of 'Equagesic' tablets

Expt. No.	Powdered sample (mg)	+Actual wt. of aspirin (A; mg)	Actual wt. of aspirin used for titration ($\frac{A}{10}$; mg)	Vol. of 0.1N $Na_2S_2O_3.5H_2O$ required by the liberated iodine (B; ml)	Vol. of 0.1N bromine required by the aspirin (50-B; ml)	Wt. of aspirin found (mg)	% labelled strength
1	1.2085	464.1	46.41	34.82	15.18	45.6	98.2
2	1.2009	450.0	45.00	35.33	14.67	44.1	97.9
3	1.2003	449.8	44.98	35.35	14.65	44.0	97.8
4	1.1905	446.1	44.61	35.42	14.58	43.8	98.3
					MEAN		98.0%
					Coeff. variation		0.53%

The nmr method is faster than the official method. For a single determination, the time involved for the determination of aspirin, meprobamate and ethoheptazine citrate by the nmr method was 30 - 40 minutes. The time for the official methods was 3 - 4 hours. However, the offical methods were more precise than the nmr method.

3.4.2 'CODIS'

'Codis' is a tablet preparation containing aspirin (500 mg) and codeine phosphate (8 mg) as the active ingredients.

The aspirin and codeine phosphate content of 'Codis' have been determined by nmr assay using *p*-*t*-butylbenzoic acid as the internal standard. In view of the low codeine phosphate content, analar codeine phosphate was added to the sample to increase the sensitivity. The results are given in table 3:39, page 265.

The official assay method[153] was used to determine the aspirin and codeine phosphate content. The method for codeine phosphate is an acid-base titration. The codeine base was generated from the phosphate proton salt and treated with standard sulphuric acid. The excess sulphuric acid was determined by titrating with sodium hydroxide. The results for both determinations (aspirin and codeine phosphate) are given in tables 3:40 and 3:41, pages 267 and 268.

The results obtained by both methods (nmr and official) agreed except that the coefficient of variation for the nmr method was higher than for the official method.

Table 3:39 NMR assay of 'Codis'

Av. wt. of a tablet = 783.9 mg

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered tablets taken(A;mg)	CODEINE PHOSPHATE Standard codeine phosphate added(mg)	Av. integrals (cm) I_s	I_T	Total wt. of codeine phosphate found(mg)	Wt. of codeine phosphate found in A (mg)	+Actual wt. of codeine phosphate in A(mg)	% labelled strength
1	80.2	764.5	85.8	4.69	1.56	91.05	7.25	7.80	92.9
2	79.9	765.0	84.0	4.66	1.56	91.62	7.62	7.81	97.6
3	80.1	780.0	85.5	4.69	1.59	92.91	7.42	7.96	93.2
4	80.2	777.2	84.9	4.68	1.58	92.51	7.55	7.93	95.2
5	79.8	785.0	86.4	4.65	1.60	94.00	7.56	8.01	94.4
							MEAN		94.7%
							Coeff variation		2.00%

+Based on the labelled strength

Table 3:39 (contd.) Aspirin

Expt. No.	Wt. of internal standard (mg)	Wt. of powdered tablets taken (A; mg)	Av. integrals (cm) I_S	I_T	ASPIRIN +Actual wt. of aspirin (mg)	Wt. of aspirin (mg)	% labelled strength
1	80.2	764.5	4.69	10.30	487.67	534.0	109.5
2	79.9	765.0	4.66	10.18	488.02	529.5	108.5
3	80.1	780.0	4.69	9.85	497.56	510.0	102.5
4	80.2	777.2	4.68	9.89	495.76	514.1	103.7
5	79.8	785.0	4.65	9.80	500.69	510.2	101.9
					MEAN		105.2%
					Coeff. variation		3.38%

+ Based on the labelled strength

Table 3:40 Official assay method[153] for the aspirin content of 'Codis'

Expt. No.	Powdered sample (mg)	+Actual wt. of aspirin (A; mg)	Actual wt. of aspirin used for titration ($\frac{A}{10}$; mg)	Vol. of 0.1N $Na_2S_2O_3.5H_2O$ required by liberated iodine (B; ml)	Vol. of 0.1N bromine reqd. by the aspirin (50-B; ml)	Wt. of aspirin found (mg)	% labelled strength
1	784.8	500.57	50.06	33.08	16.92	50.8	101.5
2	783.9	500.0	50.00	33.15	16.85	50.6	101.3
3	789.0	503.25	50.32	32.95	17.05	51.2	101.8
4	787,5	502.30	50.23	33.02	16.98	51.0	101.6
					MEAN		101.6%
					Coeff. variation		0.44%

Table 3:41 Official assay method[153] for codeine phosphate content of 'Codis'

Expt. No.	Powdered sample (mg)	+Actual wt. of codeine phosphate (mg)	Vol. of 0.02N NaOH used to neutralise the excess acid (B; ml)	Vol. of 0.02N HCl required by codeine (5-B; ml)	Wt. of codeine phosphate found (mg)	% labelled strength
1	1.0081	10.29	3.81	1.19	9.7	94.3
2	1.0950	11.17	3.71	1.29	10.5	93.9
3	1.2000	12.25	3.57	1.43	11.6	95.0
4	1.0990	11.22	3.70	1.30	10.6	94.9
					MEAN	94.5%
					Coeff. variation	0.79%

3.4.3 'CAFADOL'

'Cafadol' is a tablet preparation containing paracetamol (500 mg) and caffeine citrate (30 mg) as the active ingredients.

An nmr method of determination of paracetamol and caffeine citrate has been carried out using *p*-*t*-butyl benzoic acid as the internal standard, and pure caffeine citrate to raise the nmr signal sensitivity of the caffeine citrate content in the tablet. As with 'Codis' (section 3.4.2, page 264) the caffeine citrate content of 'Cafadol' is low compared in relation to the paracetamol. Addition of analar caffeine citrate is necessary. The results are given in table 3:42, page 270.

Despite the low content of caffeine citrate in a tablet, the result obtained is quite satisfactory. It appears that for low content ingredients, the use of the pure drug to increase the sensitivity can give satisfactory results.

The paracetamol content complies with the official requirements (95.0 - 105.0%).

3.4.4 'PARAHYPON'

'Parahypon' is a tablet preparation containing the following active ingredients per tablet:

Paracetamol	500 mg
Caffeine	10 mg
Codeine phosphate	5 mg

Table 3:42 NMR assay of 'Cafadol' Paracetamol

Av. Wt. of a tablet = 598.8 mg

Expt. No.	Wt. of internal standard (mg)	Av. Integral (cm) I_s	wt. of powdered sample (A; mg)	Av. integral (cm) I_T	PARACETAMOL +Actual wt. of paracetamol in A (mg)	wt. of paracetamol found in A (mg)	% labelled strength
1	88.0	9.00	312.2	10.11	260.56	251.7	96.6
2	86.5	9.01	312.2	10.69	260.68	261.2	100.2
3	87.2	9.03	309.6	10.48	258.63	257.6	99.6
4	86.9	9.00	313.1	10.20	261.59	250.6	95.8
5	86.0	9.00	309.5	10.37	258.40	252.2	97.6
6	87.5	9.05	311.9	10.16	260.42	250.0	96.0
7	87.2	9.01	304.6	10.12	254.29	249.2	98.0
8	86.3	9.00	288.1	9.84	240.68	240.2	99.8
					MEAN		98.0%
					Coeff. variation		1.79%

+Based on the labelled strength

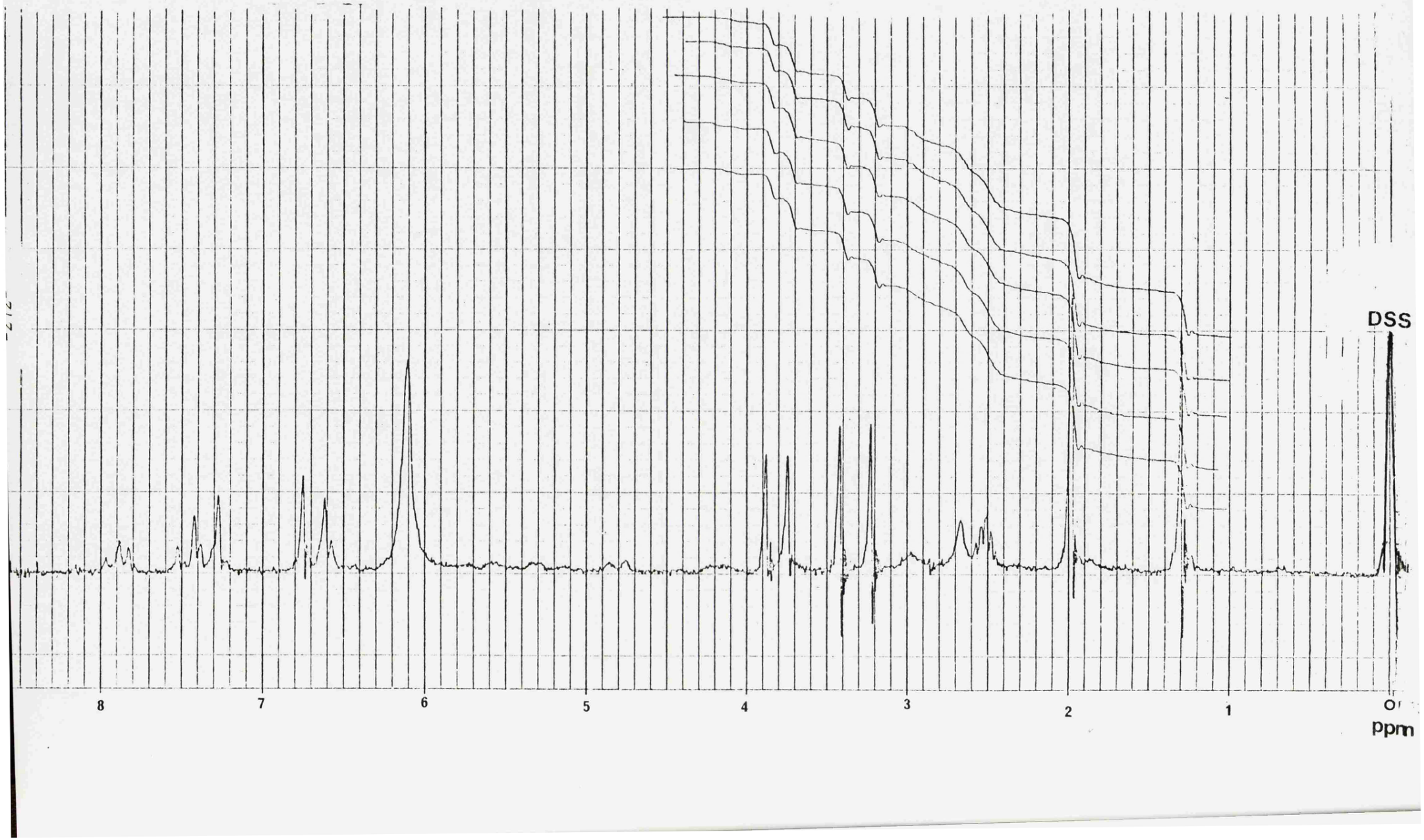
Table 3:42 (contd.) Caffeine citrate

Expt. No.	Wt. of internal standard (mg)	Av. integral (cm) I_s	wt. of powdered sample (A;mg)	Standard caffeine citrate added (mg)	Average integral (cm) I_T	CAFFEINE CITRATE Total Wt. of caffeine citrate found	Wt. of caffeine citrate found in A (mg)	+Actual wt. of caffeine citrate in A(mg)	% labelled strength
1	88.0	9.00	312.2	62.0	1.23	78.5	16.5	15.62	105.6
2	86.5	9.01	312.2	64.2	1.30	81.2	17.0	15.61	108.9
3	87.2	9.03	309.6	60.1	1.23	77.2	17.1	15.52	110.2
4	86.9	9.00	313.1	61.1	1.23	77.0	15.9	15.73	101.1
5	86.0	9.00	309.5	62.5	1.26	78.0	15.5	15.53	99.8
6	87.5	9.05	311.9	61.4	1.24	78.0	16.6	15.66	106.0
7	87.2	9.01	304.6	63.2	1.25	78.5	15.3	13.85	110.5
8	86.3	9.00	288.1	59.9	1.20	74.7	14.8	14.41	102.7
						MEAN			104.5%
						Coeff Variation			3.76%

+Based on the labelled strength

-271-

FIG 3:5 Assay of 'Parahypon'



The active ingredients of 'Parahypon' have been determined using *p*-*t*-butylbenzoic acid as the internal standard. In view of the low caffeine and codeine phosphate contents, analar caffeine and analar codeine phosphate were added to raise the sensitivity level of the two substances in the tablet. The results obtained from this analysis are given in table 3:43, page 274 . The nmr spectrum for the assay of 'Parahypon' is given in figure 3:5, page 272.

The results for the paracetamol and caffeine were satisfactory but for codeine phosphate unsatisfactory results were obtained. The average % labelled strength obtained for paracetamol, caffeine and codeine phosphate were 96.6, 94.2 and -1.6 respectively. The poor results obtained for codeine phosphate indicates that there is a limit to the quantity of codeine phosphate per tablet suitable for nmr analysis, even when analar codeine phosphate is added.

3.4.5 'FRANOL' EXPECTORANT

'Franol' expectorant contains the following active ingredients per 5 ml.

Phenobarbitone	4 mg
Ephedrine	4.75 mg
Theophylline monohydrate	60.0 mg
Guaiphenesin	25.0 mg

Table 3:43 NMR assay of 'Parahypon' Paracetamol

Av. wt. of a tablet = 613.2 mg

Expt. No.	wt. of internal standard (mg)	Av. integral (cm) I_S	wt. of powdered sample (A; mg)	Av. integral (cm) I_T	PARACETAMOL +Actual wt. of paracetamol in A (mg)	wt. of paracetamol found in A (mg)	% labelled strength
1	62.4	8.52	245.7	10.58	200.30	197.3	98.5
2	64.5	8.57	266.1	10.48	217.0	208.5	96.1
3	63.2	8.54	256.2	10.25	208.87	193.0	92.4
4	62.1	8.50	239.5	10.22	195.27	190.0	97.3
5	63.8	8.56	277.1	11.74	225.96	222.8	98.6
6	63.4	8.55	265.7	11.12	216.61	209.9	96.9
					MEAN		96.6%
					Coeff. variation		2.36%

Table 3:43 (contd.) Caffeine

Expt. No.	Wt. of internal standard (mg)	Av. integral (cm) I_s	Wt. of powdered sample (A;mg)	Wt. of standard caffeine added(mg)	Average integral (cm) I_T	Total wt. of caffeine found (mg)	Wt. of caffeine found in A (mg)	+Actual wt. of caffeine in A (mg)	% Labelled strength
					CAFFEINE				
1	62.4	8.52	245.7	96.8	4.20	100.5	3.7	4.00	92.5
2	64.5	8.57	266.1	95.2	4.04	99.5	4.3	4.57	94.1
3	63.2	8.54	256.2	97.0	4.17	100.9	3.9	4.17	93.6
4	62.1	8.50	239.5	93.5	4.07	97.3	3.8	3.89	97.8
5	63.8	8.56	277.1	90.1	3.87	94.4	4.3	4.52	95.1
6	63.4	8.55	265.7	90.7	3.91	94.7	4.0	4.35	91.9
							MEAN		94.2
							Coeff variation		2.24%

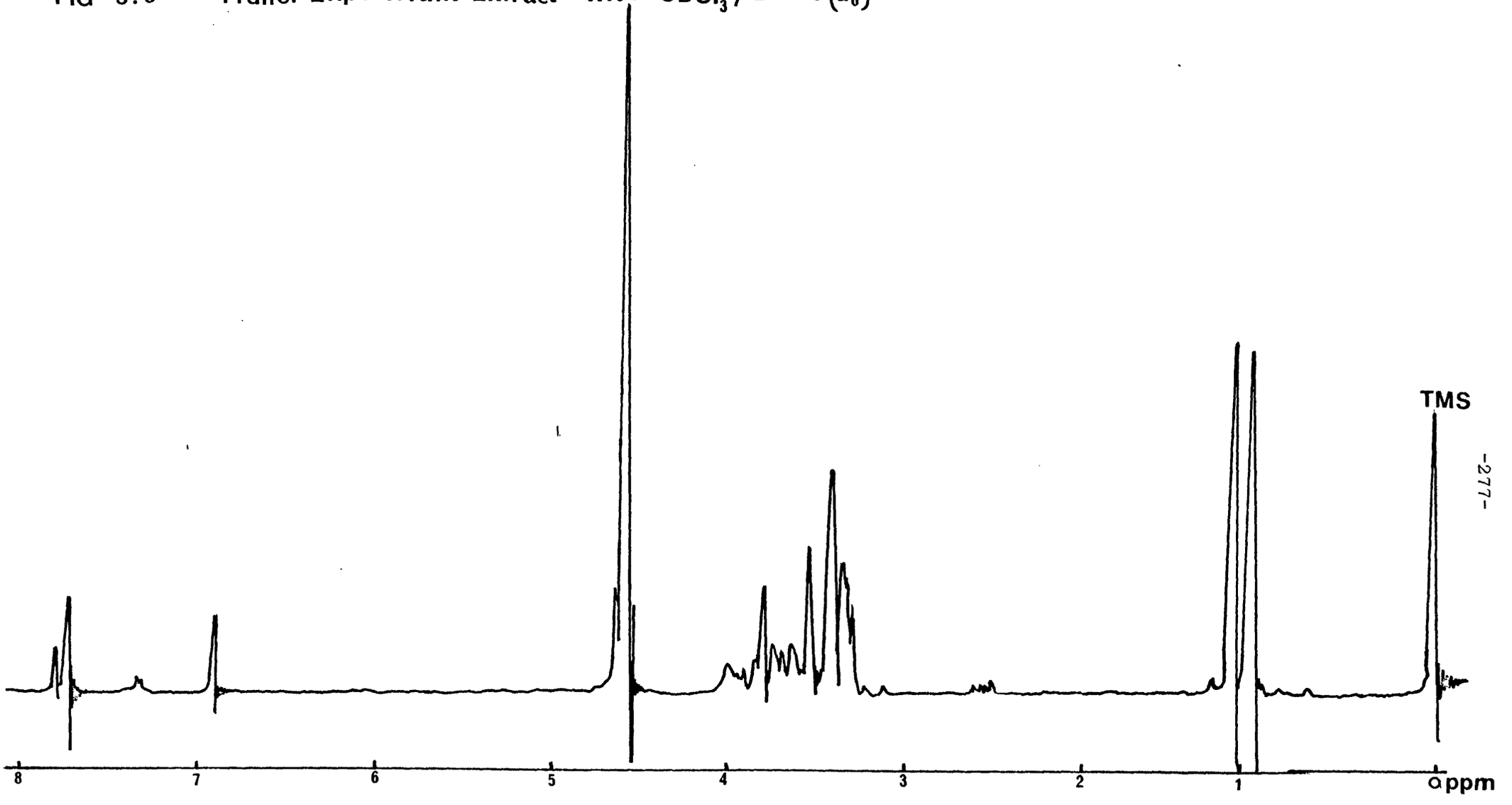
+Based on the labelled strength

Table 3:43 (contd.) Codeine phosphate

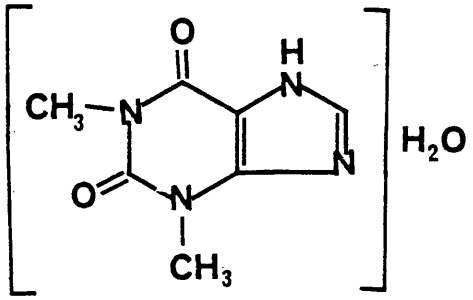
Expt. No.	CODEINE PHOSPHATE wt. of standard codeine phosphate added (mg)	Av. integral (cm) I_T	Total wt. of codeine phosphate found (mg)	Wt. of codeine phosphate found in A (mg)	+Actual wt. of codeine phosphate in A (mg)	% labelled strength
1	100.1	2.00	100.0	-0.10	2.00	-5.0
2	101.1	1.96	101.0	-0.10	2.17	-4.6
3	100.2	1.98	100.2	0.00	2.09	0.0
4	99.9	1.92	99.9	0.00	1.95	0.0
5	101.5	1.99	101.5	0.00	2.26	0.0
6	101.1	1.99	101.1	0.00	2.17	0.0
				MEAN		-1.6%
				Coeff. variation		1.14%

+ Based on the labelled strength

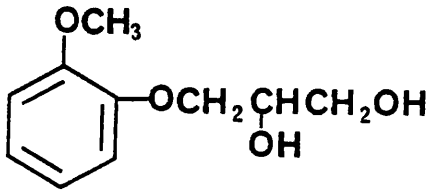
FIG 3:6 'Franol' Expectorant Extract with $CDCl_3$ / DMSO(d_6)



An attempt to use nmr to assay this preparation failed. The phenobarbitone and the ephedrine contents are too low to be assayed accurately by nmr, but the theophylline monohydrate (3:7) and the guaiphenesin (3:8) contents are present in sufficient quantity for nmr analysis. The nmr spectrum of the chloroform extract of the preparation (figure 3:6, page 277) revealed the presence of additional resonance from excipients, which precluded quantification of guiaphenesin and theophylline monohydrate.



(3:7)



(3:8)

Table 3:44 Summary of the conditions for the assay of formulations in section 3.4

Formulation	Active ingredient	Equivalent wt. (E_T)	Sample analytical group	Resonance position (ppm)	Internal standard	Solvent	(Vol; ml)
Equagesic	Meprobamate	54.60	$2(CH_2O)$	3.8	Meprobamate	Acetone(d_6)	(2)
	Aspirin	60.05	$COCH_3$	2.2			
	Ethoheptazine citrate	90.70	Aromatic protons	Aromatic position	Maleic acid	DMSO(d_6)	(0.7)
Codis	Aspirin	60.05	$COCH_3$	2.2	*p*-*t*-butyl-benzoic acid	DMSO(d_6)	(1)
	Codeine phosphate	67.73	OCH_3 + 3 other protons	3.5-4.2			
Cafadol	Paracetamol	50.40	$NCOCH_3$	1.9-2.1	*p*-*t*-butyl-benzoic acid	DMSO(d_6)	(1.5)
	Caffeine citrate	128.76	N^3-CH_3	3.1-3.3			
Parahypon	Paracetamol	50.40	$COCH_3$	2.0	*p*-*t*-butyl-benzoic acid	DMSO(d_6)	(1.5)
	Caffeine	64.73	N^3-CH_3	3.25			
	Codeine phosphate	135.46	OCH_3	3.75			

3.5 CONCLUSION

NMR is a technique suitable for quantitative analysis of pharmaceuticals. The nmr method reported here has the advantage over most existing methods of speed, specificity, sample size and simplicity. NMR affords accurate analysis comparable with the existing methods, although the precision is not so great.

The suitability of the nmr method for quantitative pharmaceutical analysis depends on the method of measurement of the analytical signals. Integration has been found more suitable than peak height method.

3.6 EXPERIMENTAL

NMR spectra were recorded on a Perkin Elmer R12B instrument operating at 60 MHz, with a probe temperature of $37^{\circ} \pm 1^{\circ}C$. Except where the solvent was $CDCl_3$, D.S.S. was used as internal reference standard. With $CDCl_3$ as solvent, TMS was the internal reference standard.

UV absorbances were measured on a Perkin Elmer 124 instrument.

Chemicals used were obtained from the following sources:

Analar maleic acid was purchased from the British Drug House. All other internal standards mentioned in table 3:1A (page 201) were purchased from Aldrich Chemical Co. Ltd.

Gentamicin sulphate samples were supplied by the Nicholars Laboratories Ltd.

Ethoheptazine citrate and meprobamate were supplied by John Wyeth & Brother Ltd., Maidenhead.

Acetylsalicylic acid was purchased from Evans Chemical Co., Bristol.

All the tablets, capsules and suspensions were purchased from M.I. and J.T.R. Owen Chemists, Bristol.

3.6.1 Gentamicin Analysis

3.6.1.1 Gentamicin sulphate assay

3.6.1.1.1 Gentamicin sulphate powder

Gentamicin sulphate (*Ca* 0.09 g) and maleic acid (*Ca* 0.05 g) were dissolved in D_2O (0.5 ml). The nmr spectrum was recorded and the appropriate signals integrated several times.

3.6.1.1.2 Gentamicin injection

2 ml of each ampoule were pipetted into small beakers and left in an oven at a temperature of 40°C and a pressure below 5 mm Hg to remove solvent. The residue, together with maleic acid (*Ca* 0.04 g), was then dissolved in D_2O (0.5 ml). The nmr spectrum was recorded and the appropriate signals integrated.

3.6.1.2 Chromatographic separation of the major components of the gentamicin complex

The method of Wagman, Marquez and Weinstein[164] was followed.

Adsorbent	- Cellulose (Whatman cellulose powder std. grade
Mobile phase	- Lower layer of a mixture of chloroform, methanol and 17% ammonia (2:1:1).
Length of column	- 40 cm.
Width	- 2 cm.
Rate of flow	- 20 ml./15 min.
Sample	- Gentamicin sulphate powder (4.0 g) Batch No. GMC 4-X-6007.

The Gentamicin sulphate was mixed with cellulose (20 g). The mixture was placed on top of the packed column and moistened with a few ml. of the upper layer of the chloroform, methanol and 17% ammonia mixture.

Fractions were collected at 10 -15 min intervals.

Component	Fraction No.	Weight (mg)
C_1	3-6	198.6
C_2	10-17	103.9
C_{1a}	30-39	59.7

The gentamicins were detected by descending paper chromatography[164].

3.6.2 Pharmaceutical preparations containing one active ingredient

3.6.2.1 NMR assay of tablets

The average weight of a tablet was obtained from 20 tablets. The tablets were powdered and a known weight of the powder suspended in the appropriate solvent together with the internal standard (see table 3:33, page 254). The mixture was allowed to stand for at least 5 mins. with occasional shaking to ensure complete dissolution. The mixture was either centrifuged or filtered through a cotton wool plug saturated with the solvent. The nmr spectrum of the clear supernatant layer or the filtrate was recorded and the analytical regions (see tables 3:1, page 201 and 3:33), integrated at least five times.

3.6.2.2 NMR Assay of suspensions

(see tables 3:11, page 223 and 3:30, page 251 for details of the quantities of the suspension and internal standard used).

A sufficient quantity of the suspension was mixed with the internal standard and the solvent (table 3:33). The mixture was stirred for about 10 minutes, then centrifuged or filtered through a cotton wool plug already saturated with the solvent. The nmr spectrum of the clear layer or filtrate was recorded and the analytical regions integrated several times.

3.6.2.3 NMR assay of capsules

(see tables 3:16, page 229, 3:23, page 242 and 3:33, page 254 for the quantities of samples, internal standards and solvents employed).

The contents of ten capsules were weighed together and a known weight equivalent to a sufficient quantity of the active ingredient suspended in the appropriate solvent, together with the internal standard. The mixture was either filtered or centrifuged after occasional shaking for 5 minutes. The nmr spectrum of the filtrate or the supernatant layer was recorded and the analytical peaks integrated.

3.6.2.4 UV assay of 'Orudis' capsules

The contents of ten capsules were weighed. The weight of the powder equivalent to 50 mg of ketoprofen was suspended in methanol (*Ca* 50 ml). It was then filtered into a 200 ml volumetric flask and the volume made up with methanol. 5 ml. of the solution was

diluted to 100 mls with methanol. The absorbance in a 1 cm layer of the resulting solution at 253 nm was recorded. The absorbance (0.89) at 253 nm of a standard ketoprofen solution (0.00131%, w/v) in methanol was employed for the calculation.

3.6.2.5 UV assay of 'Brufen' suspension

A weight of the suspension equivalent to about 20 mg of ibuprofen (table 3:13, page 225) was mixed with methanol (20 ml) and stirred for 10 minutes. The mixture was then filtered. The filtrate was diluted with methanol (to 100 ml) and 5 ml of the resulting solution further diluted with methanol (to 100 ml). The extinction of the final solution in 1 cm layer was measured at 222 nm. The extinction (0.430) of a standard ibuprofen solution (0.00103%, w/v) in methanol at 222 nm was used for the calculation.

3.6.2.6 UV assay of 'Brufen' tablets

A weight of powdered tablet equivalent to about 27 mg of ibuprofen (table 3:12, page 224) was suspended in methanol (30 ml) and stirred for 10 minutes. The mixture was then filtered. The filtrate was diluted with methanol (to 100 ml) and 5 ml of the resulting solution further diluted with methanol (to 100 ml). The extinction of the final solution in 1 cm layer was measured at 222 nm. The extinction (0.430) of a standard ibuprofen solution (0.00103%, w/v) in methanol at 222 nm was used for the calculation.

3.6.2.7 UV assay of 'Naprosyn' tablets

A weight of powdered tablet equivalent to about 20 mg of naproxen (table 3:28, page 248) was suspended in methanol (30 ml),

stirred for 10 minutes and then filtered. The filtrate was diluted with methanol (to 100 ml). 5 ml of the resulting solution was further diluted with methanol (to 50 ml). The extinction of the final solution in a 1 cm layer was measured at 273 nm. The absorbance (0.532) of a standard naproxen solution (0.00265%, w/v) in methanol at 273 nm was employed for the calculation.

3.6.2.8 UV assay of 'Benoral' tablets

About 22 mg of powdered tablet (see table 3:31, page 252) was dissolved in hot methanol (20 ml) and then diluted with methanol (to 100 ml). 5 ml of the solution was further diluted with methanol (to 200ml). The absorbance of the resulting solution at 240 nm in a 1 cm layer was recorded. The absorbance (0.410) of a standard benorylate solution (5.175×10^{-4}%, w/v) in methanol at 240 nm was used for the calculation.

3.6.2.9 UV assay of 'Benoral' suspension

A quantity of the suspension equivalent to 20 mg of benorylate (table 3:32, page 253) was mixed with hot methanol (30 ml) and stirred for 5 minutes. The mixture was filtered and diluted with methanol (to 100 ml). 5 ml of the solution was further diluted with methanol (to 200 ml). The absorbance of the resulting solution at 240 nm in a 1 cm layer was recorded. The absorbance (0.410) of a standard benorylate solution (5.175×10^{-4}%, w/v) in methanol at 240 nm was used for the calculation.

3.6.3 Assay of formulations containing more than one active ingredient

3.6.3.1 NMR assay of 'Equagesic' tablets

3.6.3.1.1 Meprobamate and aspirin determination

20 tablets were weighed and powdered. Two equal weights of the powdered tablets (*Ca* 250 mg each; table 3:34, page 259) were taken. Pure meprobamate (*Ca* 83 mg) was added to one of them. The two samples were suspended separately in acetone(d_6; 2 ml), filtered/centrifuged, and the clear solution used for the analysis. (The residue was reserved for ethoheptazine citrate determination). The nmr spectra of the two solutions were recorded and the region between δ2.0 - δ4.5 ppm integrated several times.

3.6.3.1.2 Ethoheptazine citrate determination

Maleic acid (*Ca* 40 mg; table 3:35, page 260) was added to the residue from the meprobamate/aspirin determination (section 3.4.6.1.1) and suspended in DMSO(d_6; 0.7 ml). The suspension was stirred for about 5 minutes and filtered through a cotton wool plug saturated with DMSO(d_6). The nmr spectrum of the filtrate was recorded and the region between δ6.0 and δ8.0 ppm integrated several times.

3.6.3.2 NMR assay of 'Codis', 'Cafadol' and 'Parahypon'

(see tables 3:39, page 265, 3:42, page 270 and 3:43, page 274 for the weights of the internal standard and the powdered samples; and table 3:44, page 279 for the volume of solvent used.).

The average weight of twenty tablets was found and the tablets powdered. A known weight of the powdered sample together with the internal standard and a pure compound of caffeine, caffeine Citrate or codeine phosphate, where appropriate was suspended in DMSO(d_6). The mixture was either centrifuged or filtered. The nmr spectrum

of the clear solution obtained was recorded and the appropriate regions (see table 3:44, page 274) integrated at least five times.

PART 4

4. KINETIC STUDIES ON 'BENORAL' SUSPENSION

4.1 INTRODUCTION

To obtain the shelf life of a pharmaceutical preparation, the formulation must be stored under room temperature conditions and periodically assayed until its potency has fallen to 90% or less. This is obviously tedious, uneconomical and time consuming. Accelerated stability studies at higher temperatures have proved useful in predicting in a very short time the shelf life of pharmaceutical products[185-191].

The prediction of shelf life by accelerated tests is based on the quantitative application of fundamental physicochemical principles based on the Arrhenius equation:-

$$k = A_e^{-Ea/RT} \qquad \ldots(4:1)$$

where 'k' is the specific rate of degradation of the active ingredient;

'R' is the gas constant (1.987 calories degree^{-1} mole^{-1});

'A' is a constant which can be entropy of the reaction and/or collision factors;

'Ea' is the heat of activation (activation energy)

and 'T' is the absolute temperature.

The logarithmic form of equation (4:1)

$$\log k = -(Ea/2.303R)/(1/T) + \text{constant} \qquad \ldots(4:2)$$

shows that a plot of log k against the reciprocal of the absolute temperature (1/T) gives a linear relationship, the slope of which is given by

$$-Ea/2.303R$$

The k values for the decomposition of a drug at different elevated temperatures are obtained by plotting some function of concentration against time on an appropriate graph paper (linear, semilog or log-log) depending on the order of the reaction.

For a pseudo-zero order reaction,

$$dy/dt = -K_o \qquad \ldots(4:3)$$

i.e. $$y = y_o - k_o t \qquad \ldots(4:4)$$

where y_o and y are the initial and final concentrations at zero time and time t respectively. Plots from such a relationship are linear with a gradient equal to

$$-k$$

For a pseudo-first order reaction,

$$\log y = \log y_o - \left(\frac{k}{2.303}\right)t \qquad \ldots(4:5)$$

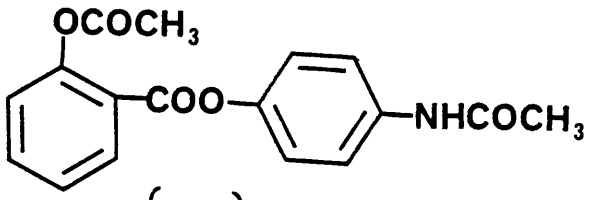
In this case the logarithmic form of the concentration is plotted against time to obtain a linear plot. The slope is given by

$$-k/2.303$$

from which 'k' can be calculated[186].

In accelerated storage testing, a function of the concentration is plotted against time at various elevated temperatures. The slopes (and hence k) for the linear plots for the various temperatures are then converted to the logarithmic form and plotted against the reciprocal of the absolute temperature. The linear plot obtained is extrapolated to the room temperature to obtain the rate constant. This procedure was adopted for the kinetic studies on 'Benoral' suspension.

'Benoral' is the proprietary name for pharmaceutical preparations containing benorylate (4-acetamidiophenyl 2-acetoxybenzoate; 2:33)



(2:33)

Benorylate is an anti-inflammatory analgesic and antipyretic used for the treatment of rheumatoid arthritis, osteoarthritis and other painful musculoskeletal conditions. It may also be used to relieve mild to moderate pain due to many other causes, and as an antipyretic in febrile conditions. 'Benoral' may only be administered orally[183].

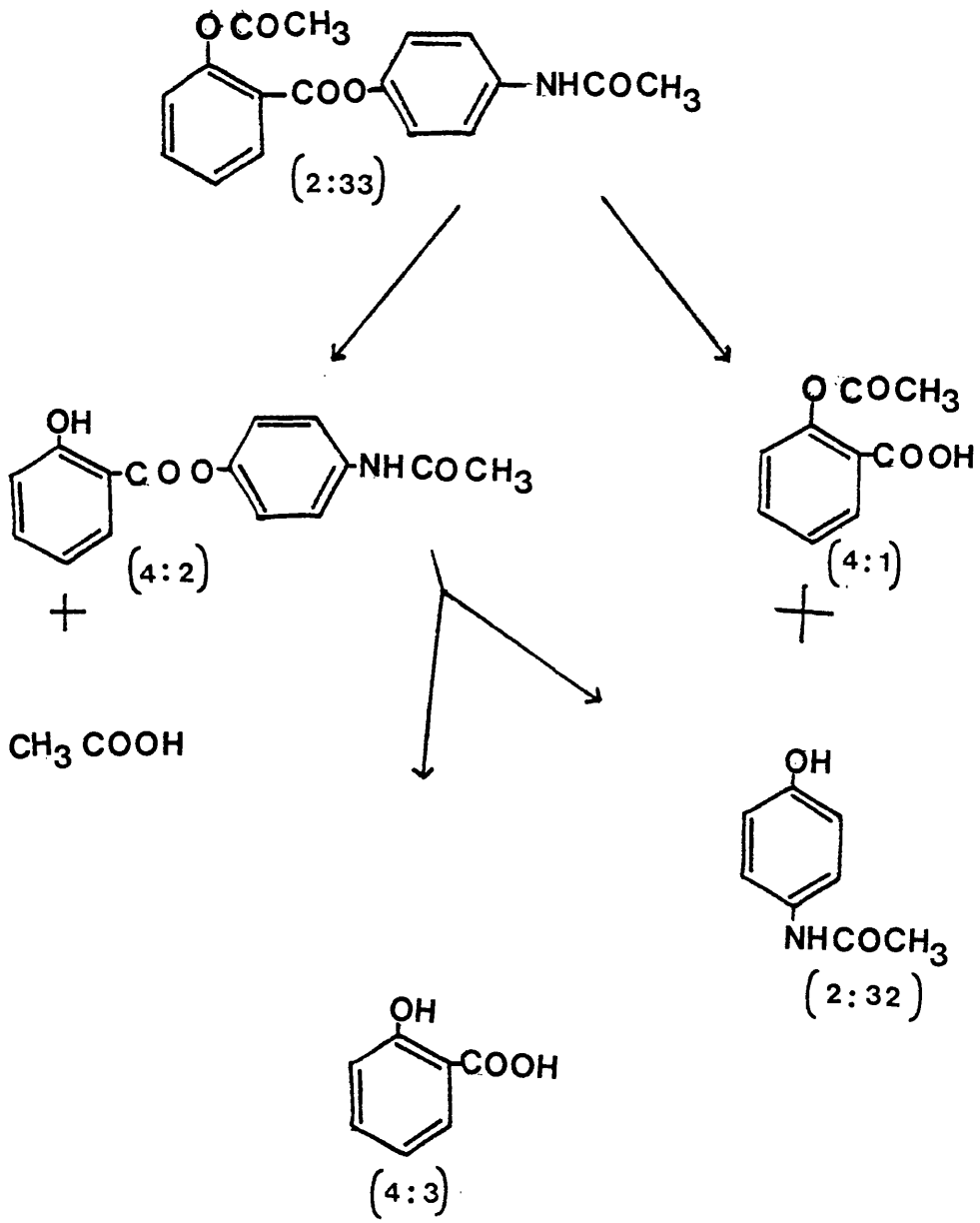
The pharmacological actions of benorylate are closely related to those exhibited by aspirin, but it has the advantage of much reduced gastric irritation[184]. The drug is degraded *in vivo* to aspirin (4:1) and paracetamol (2:32).

4.2 DISCUSSION

ACCELERATED STABILITY STUDIES ON 'BENORAL' SUSPENSION

Scheme 4:1

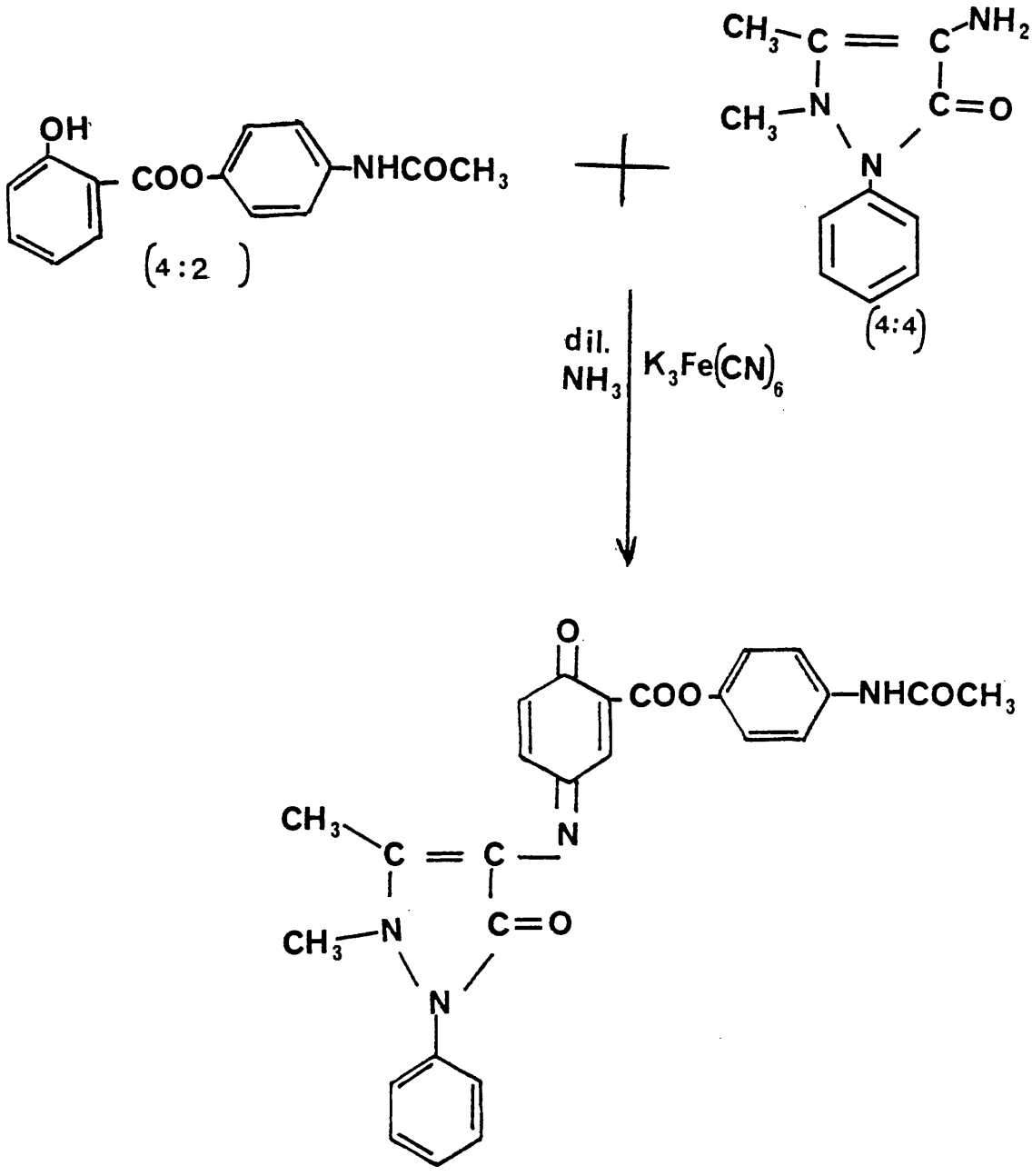
Possible Degradation Products of Benorylate



'Benoral' suspension contains 40% w/v benorylate. The stability study on this suspension was initiated since it was considered that the presence of two phenolic ester groups in the molecule might render it particularly susceptible to hydrolysis. Hydrolysis products of benorylate include paracetamol (2:32), aspirin (4:1), phenetsal (4:2), and salicylic acid (4:3) see scheme 4:1 . The presence of aspirin in the suspension would lead to gastric irritation[184].

A consideration of the structures and properties of the possible degradation products of benorylate (scheme 4:1), suggested that nmr would be an appropriate method for the stability studies, using the two resonance signals at δ2.25 ppm and δ2.10 ppm associated with the O-COCH_3 and NHCOCH_3 groups respectively. This would be possible only if the degradation product was mainly phenetsal and associated acetic acid. Since the acetic acid CH_3 resonance (δ1.99 ppm) is well separated from the signals employed for analytical purposes, and the NHCOCH_3 resonance peak - belonging to the phenetsal (δ2.05 ppm) almost coincides with that of benorylate (δ2.10 ppm), the ratio between the two resonance peaks (δ2.10/δ2.25) would be a measure of the amount of phenetsal present. On the other hand, if the degradation product consisted of paracetamol and aspirin in addition to phenetsal, the two analytical peaks (δ2.10 and δ2.25) could not be used. However, the presence of paracetamol would reveal extra peaks in the region δ6.6 to δ6.85 ppm due to two of the aromatic protons which could be used for the analysis. Unfortunately application of nmr to the problem was not successful because the amount of the degradation product present was too low to be detected on the nmr spectrometer.

Scheme 4:2



λ_{max} 480nm

The concentration of phenetsal produced at 40°C for three days was 0.15% (see tables 4:1 and 4:2, page 307) which is outside the range detectable by the instrument.

The failure of the nmr method made it necessary to find a more sensitive method. A consideration of the nature of the parent compound and the possible degradation products ruled out the use of ultraviolet (UV) or infrared (IR) spectroscopy. Since most of the degradation products are phenolic, a colorimetric method was employed for the estimation of the phenolic degradation products.

4.2.1 Colorimetric method

The reaction of a 4-aminophenazone derivative (4:4) with phenols[192] was employed for the stability study (scheme 4:2). In the presence of potassium ferricyanide in an alkaline medium, (4:4) reacts with phenols to give a coloured compound which is not stable in aqueous solution but which is stable in chloroform. The reaction is only given by phenols with the *para*- position free. Thus in a study of benorylate hydrolysis only phenetsal and salicylic acid give a coloured derivative with reagent (4:4) The colour given by both phenetsal and salicylic acid has an absorption maximum at 480 nm.

Aliquots of 'Benoral' suspension were heated at different temperatures and then analysed at time intervals (see page 314 for experimental details). The experiment was repeated using a freshly prepared suspension of benorylate in water. Also portions of the 'Benoral' suspension were buffered to different pH values and maintained at 35°C throughout the experiment. The aim of this later

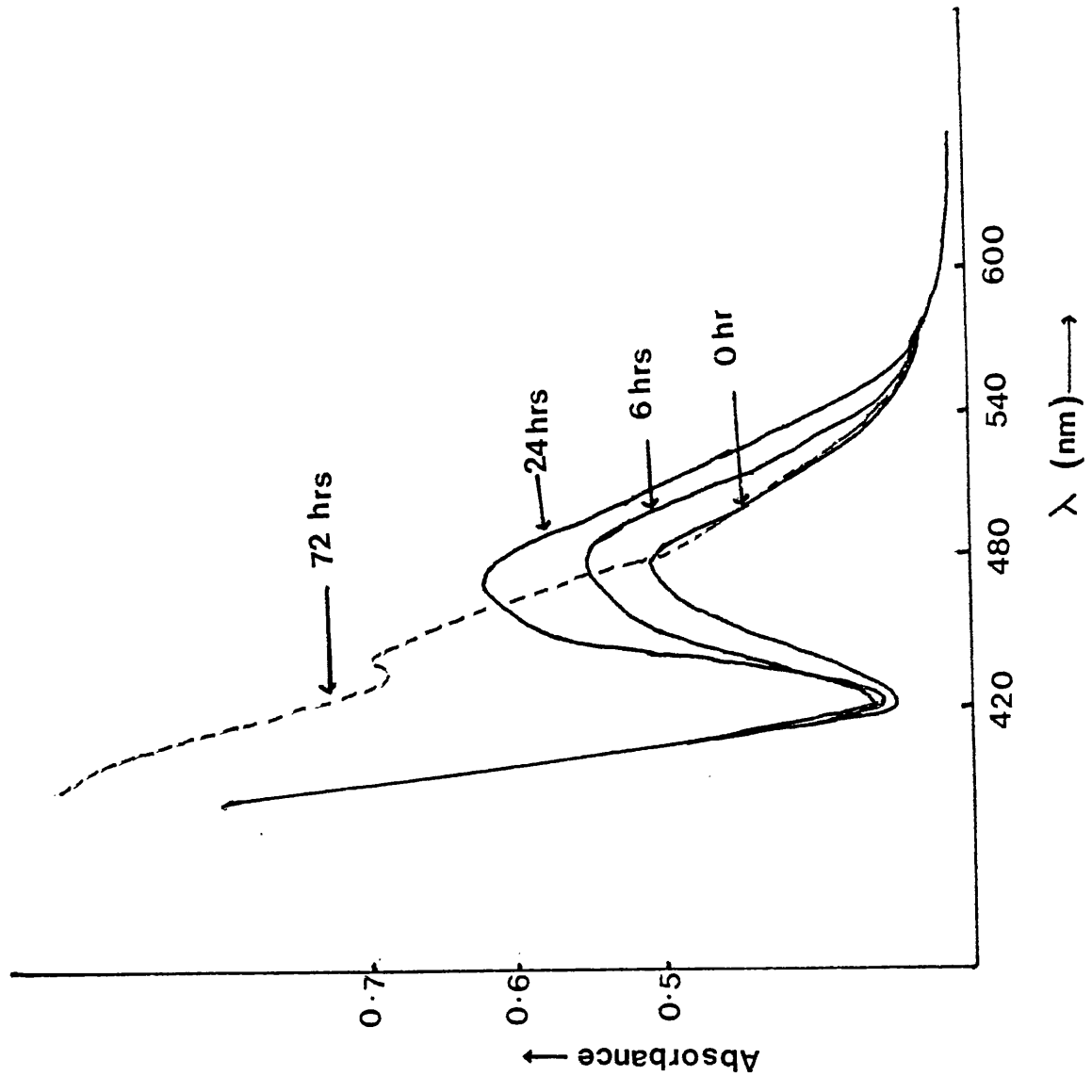


FIG. 4:1 'Benoral' Suspension at pH 8·0 and 35°C

experiment was to find the effect of pH on the stability of the suspension. Phenetsal was synthesised from salicylic acid and paracetamol (scheme 4:3)and used for calibration purposes. The results are presented in tables 4:1 to 4:7 (pages 307-312) and in figures 4:1 to 4:7 (pages 295, 301 and 303-305).

The results in table 4:2 and 4:5 (pages 308-311) show only a short linear relationship between the time and absorbance for all the various temperatures except for 30°C. A linear relationship over the entire period of the experiment was anticipated but not observed. Instead, the absorbance increased linearly with time over a short period and then decreased. These curious results initiated an investigation of the degradation pattern of benorylate. It seems that, initially the benorylate degraded to form phenetsal and acetic acid, the former yielding a colour with the 4-aminophenazone (4:4). With time, the phenetsal yielded salicylic acid and paracetamol, or the benorylate degraded to acetylsalicylic acid and paracetamol, or perhaps both occurred. The presence of paracetamol changed the absorption maximum from 480 nm to a lower wavelength, depending on the concentration of paracetamol present. Figure 4:1 (page 295) shows the absorption curves obtained for the 'Benoral' suspension buffered to pH 8 and maintained at 35°C. From figure 4:1, it is seen that there was a gradual, or essentially a linear, increase of absorbance at 480 nm with time up to 24 hours. At 72 hours, the absorbance at 480 nm fell below that at 6 hours. To prove whether this unusual absorption curve was actually due to the presence of paracetamol, 2 different weights of paracetamol were added to two, 6 ml aliquots of the 'Benoral' suspension. The absorption curves

Scheme 4:3

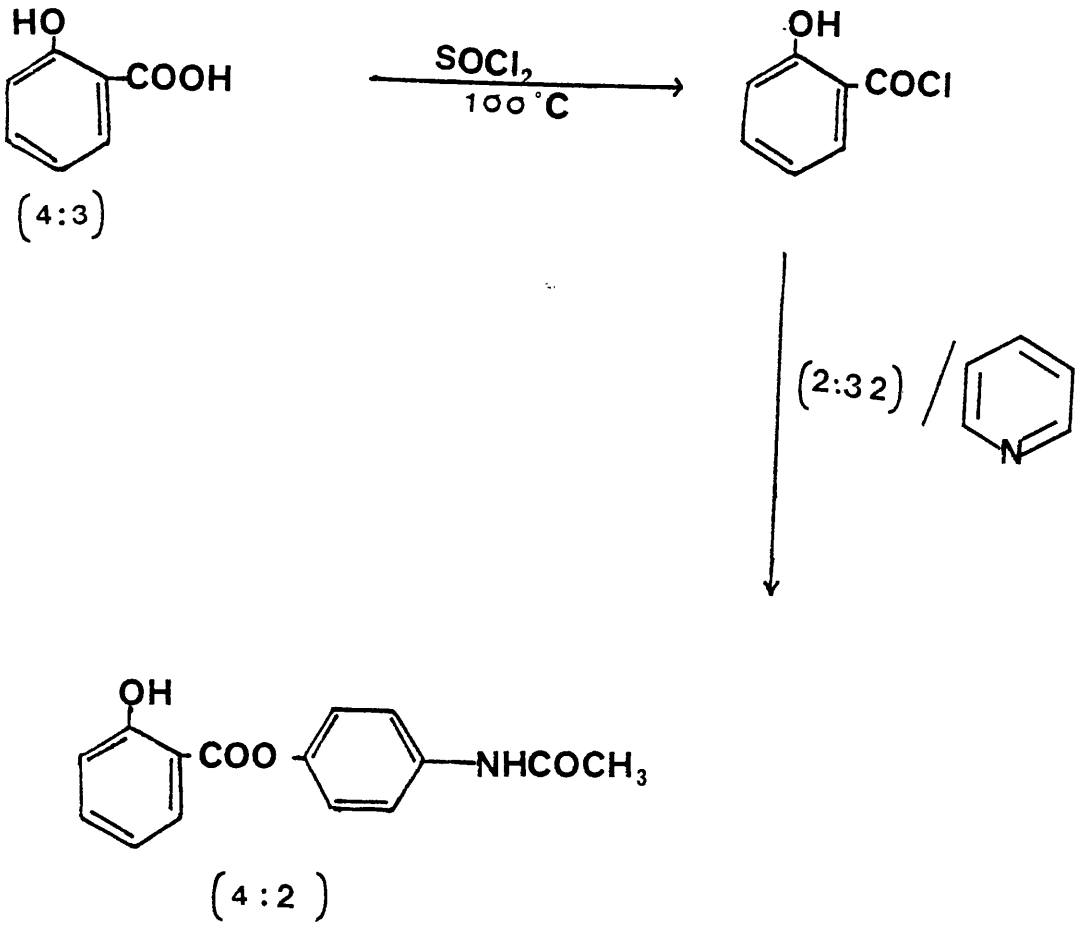
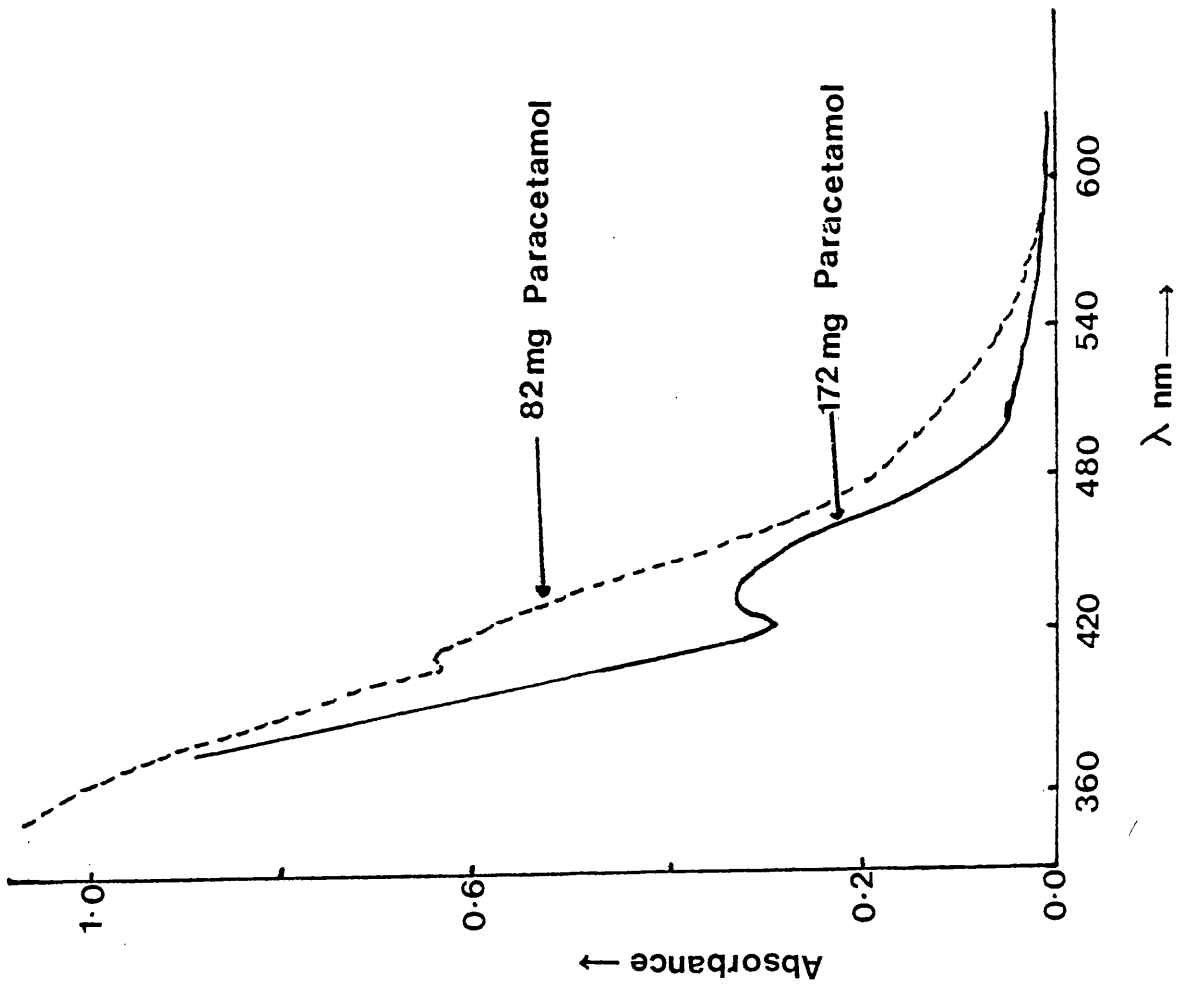


FIG. 4:2 Absorption curves of 'Benoral' Suspension with different weights of Paracetamol added at room temperature



obtained after treatment with the 4-aminophenazone (4:4) are given in figure 4:2, page 298. For comparison, 6 ml of the same 'Benoral' suspension was treated in the same way in the absence of paracetamol. The absorption curve recorded was similar to figure 4:1, 0 hour (page 295). Paracetamol itself was treated with the 4-aminophenazone reagent and the absorption curve of the resulting solution recorded (figure 4:3, page 300). There was no absorption maximum in the visible region. All these results indicated the presence of paracetamol as part of the degradation process. The shape of the absorption curve of phenetsal itself after treatment with the 4-aminophenazone (figure 4:3, page 300) is similar to that of the 'Benoral' suspension at room temperature. This confirms that the initial degradation product of benorylate is phenetsal.

Further evidence for the presence of paracetamol was obtained by treatment of the 'Benoral' suspension, at 50°C, with ferric chloride solution at 6 hour intervals. Only those suspensions which gave absorption curves similar to figure 4:2, page 298, gave a violet to blue colour. This was known after filtering the suspension through a cotton wool plug - the cotton wool became violet to blue . The experiments proved that paracetamol was not the initial degradation product, but was produced after the production of phenetsal.

4.2.2 Effect of pH on stability of 'Benoral' suspension

The stability of the 'Benoral' suspension at different pH values at a constant temperature of 35°C is shown in figure 4:4, page 301. As seen from the table of results (table 4:5, page 311) it is difficult to relate the stability of the product to the various

FIG. 4:3 Absorbance of (a) Phenetsal and (b) Paracetamol in the presence of 4— amino phenazone

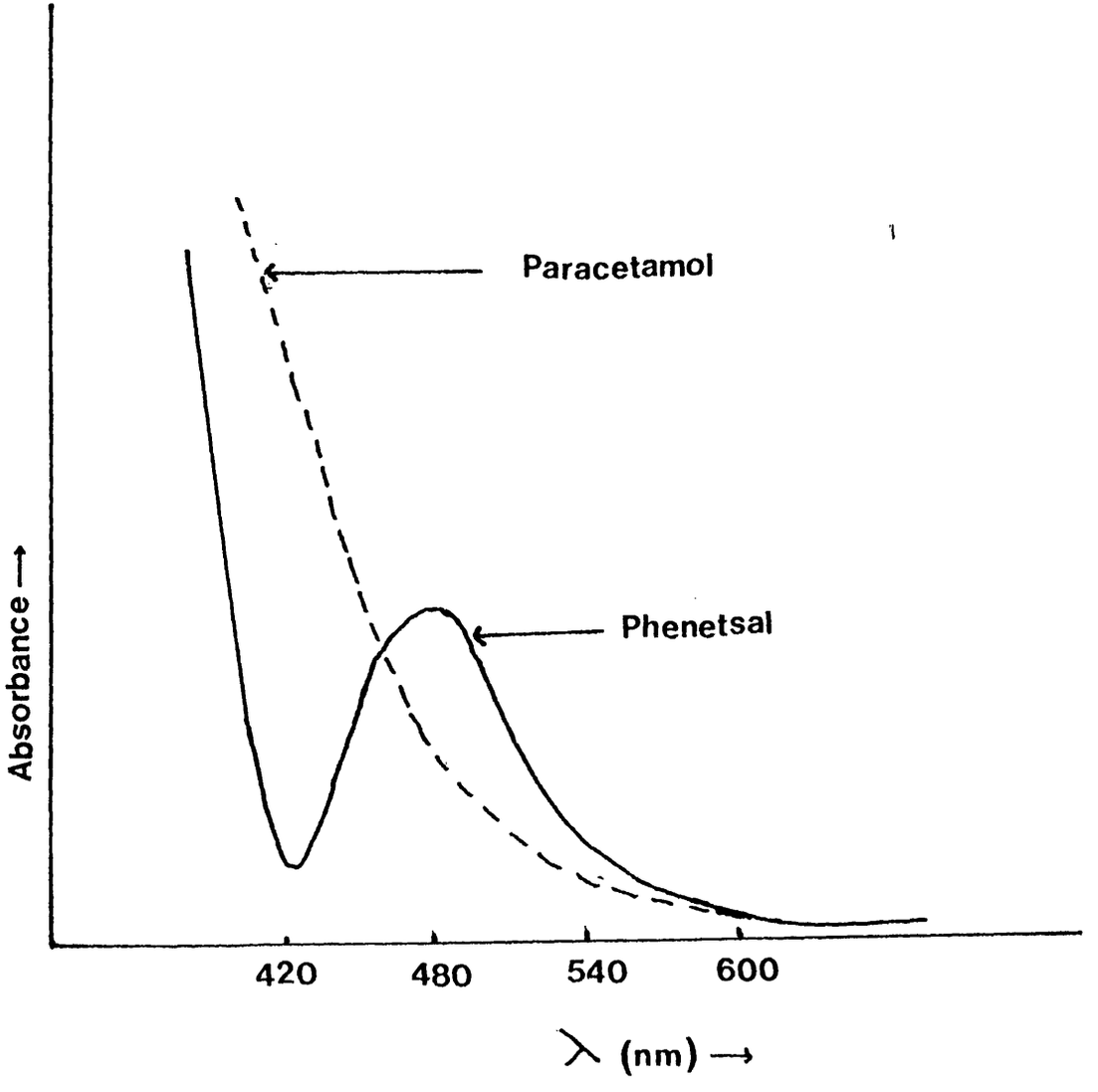
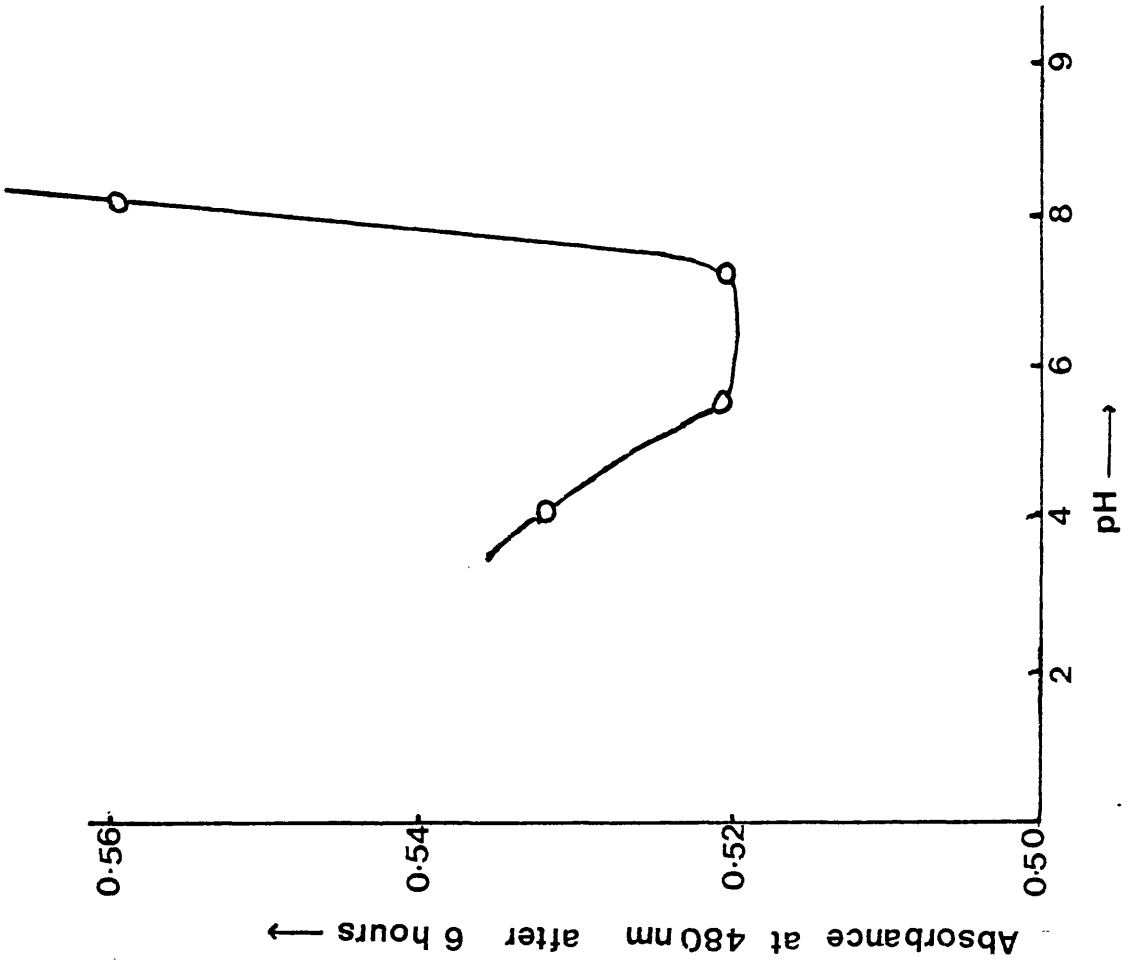


FIG. 4:4 Rate of degradation of Benorylate (in 'Benoral') at different pH

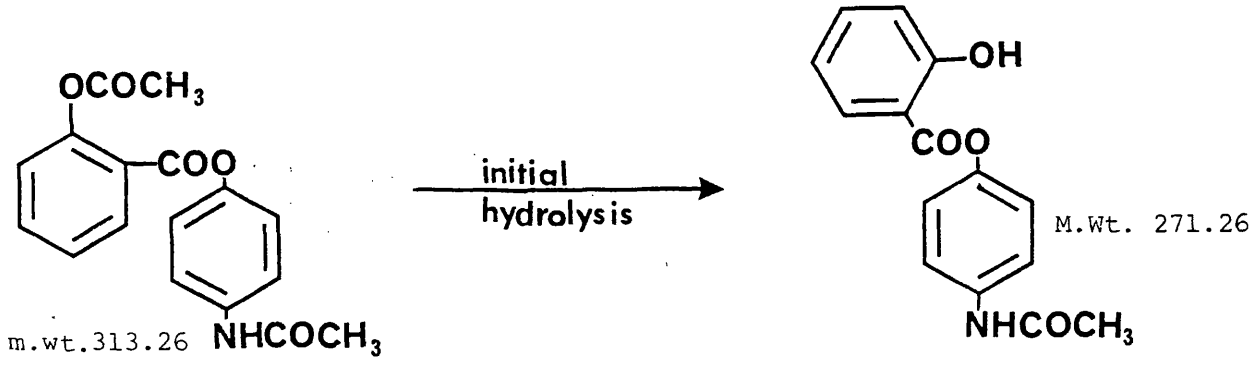


pH values over the entire period of the experiments due to the production of paracetamol. However, at or before 6 hours duration of the experiment, paracetamol had not been produced and the results up to 6 hours can therefore be used to relate the stability studies at different pH values (pH 4 - 10). It is evident from figure 4:4 that in the pH range 5 to 7.4 stability is at a maximum, Outside this range, the benorylate undergoes rapid hydrolysis. 'Benoral' suspension is formulated at pH of about 5.4 .

Table 4:6 shows the results of the stability of 'Benoral' suspension at pH 10.3. The results obtained could not be incorporated into figure 4:4 because the suspension used was from a different batch. However, the results give an indication of the instability of 'Benoral' suspension at high pH values.

4.2.3 Calculations of the shelf life at 25°C and 90% potency (t_{90})

To predict the length of time required for the 'Benoral' suspension to reach 90% potency at room temperature (25°C), the rate constant (k) at 25°C had to be obtained from the log k/1/T plot (figure 4:6, page 304). The 'k' value obtained from the graph was the absorbance increase per hour due to the production of phenetsal. The amount of phenetsal produced per hour was obtained from the calibration curve (figure 4:7, page 305). The value obtained was converted to the actual amount of benorylate degraded per hour.



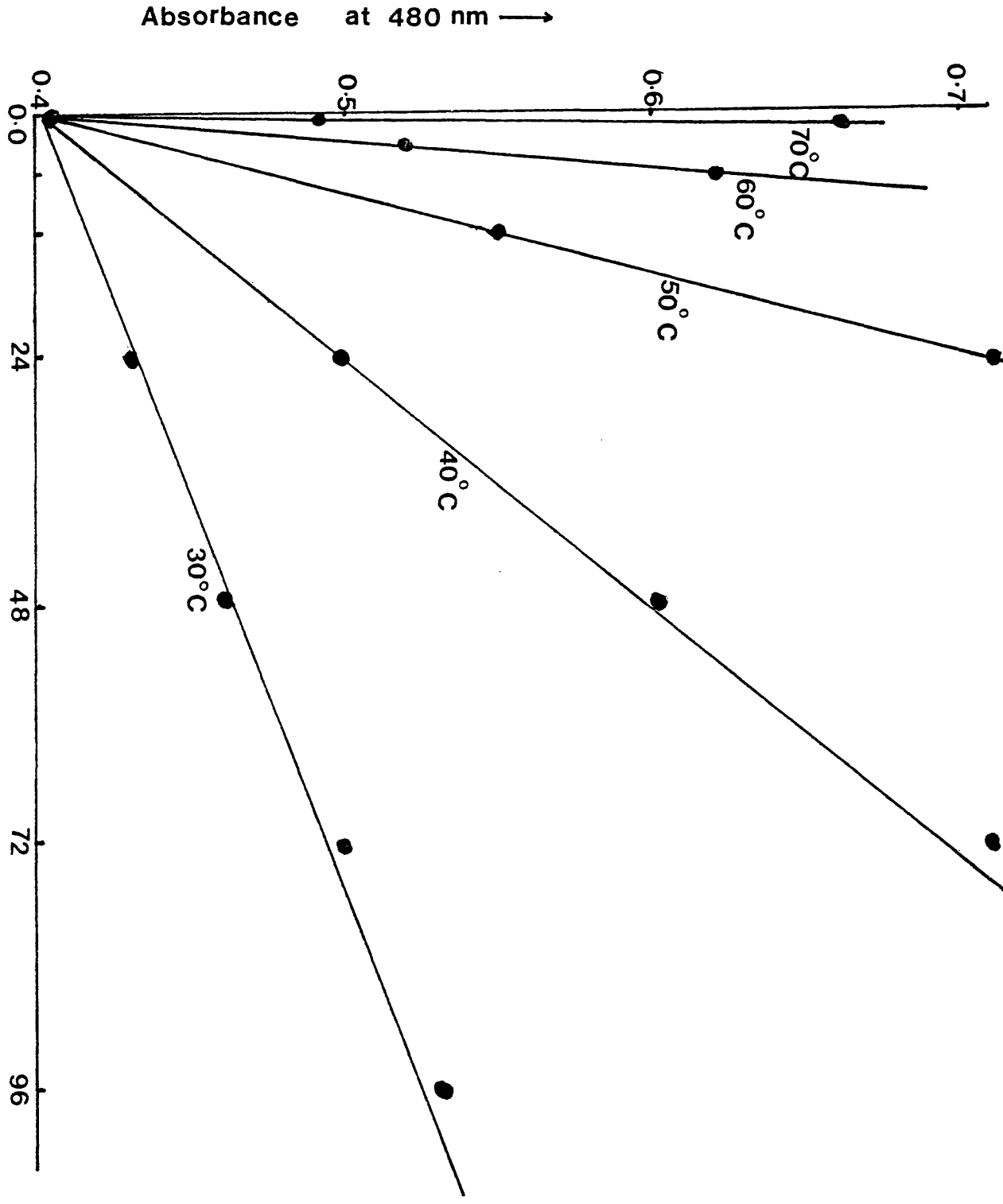
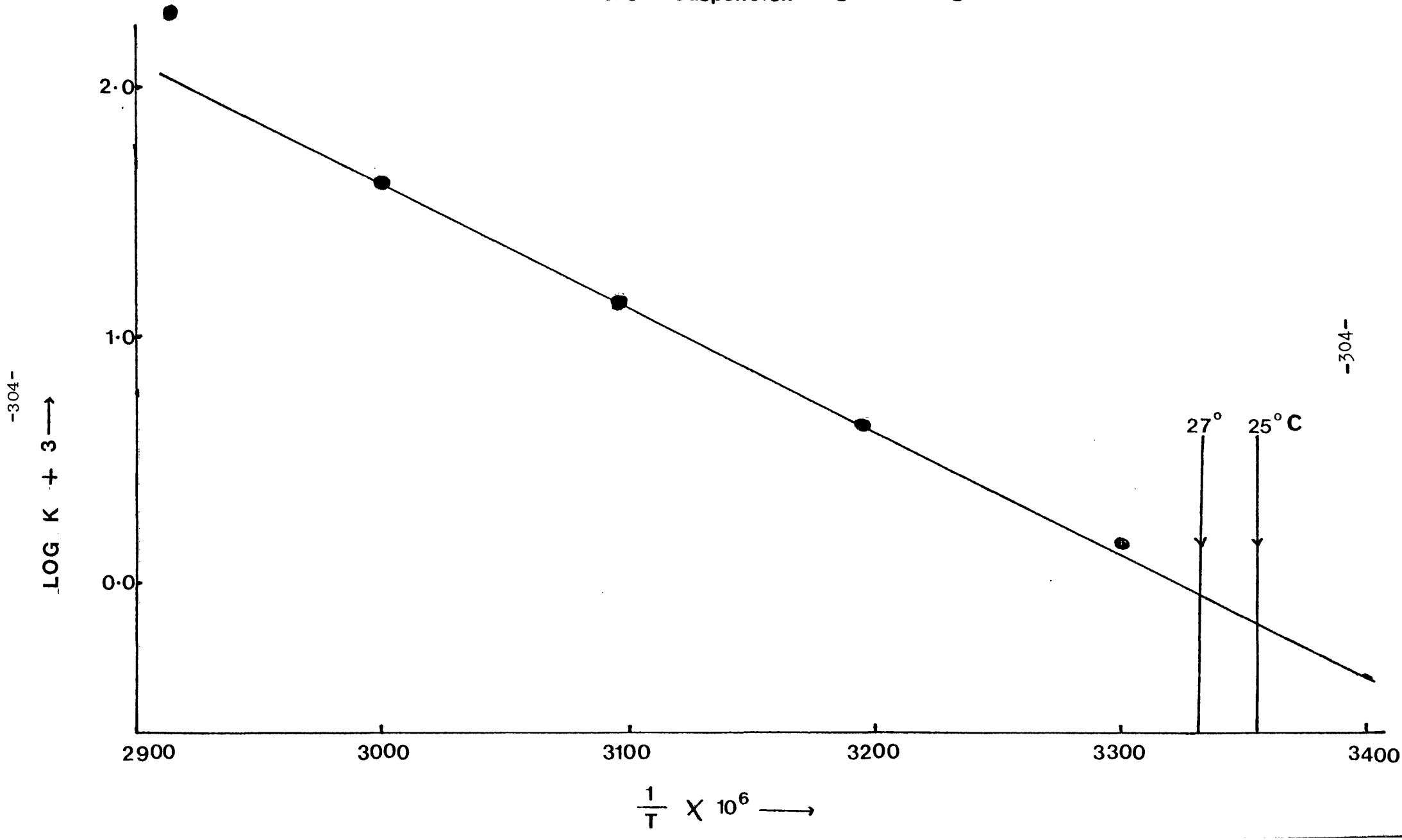


FIG. 4:5 Pseudo zero Order Plots for 'Benoral' Suspension LOT PH 106

FIG. 4:6 Arrhenius Plot for Benoral Suspension LOT PH 106



-304-

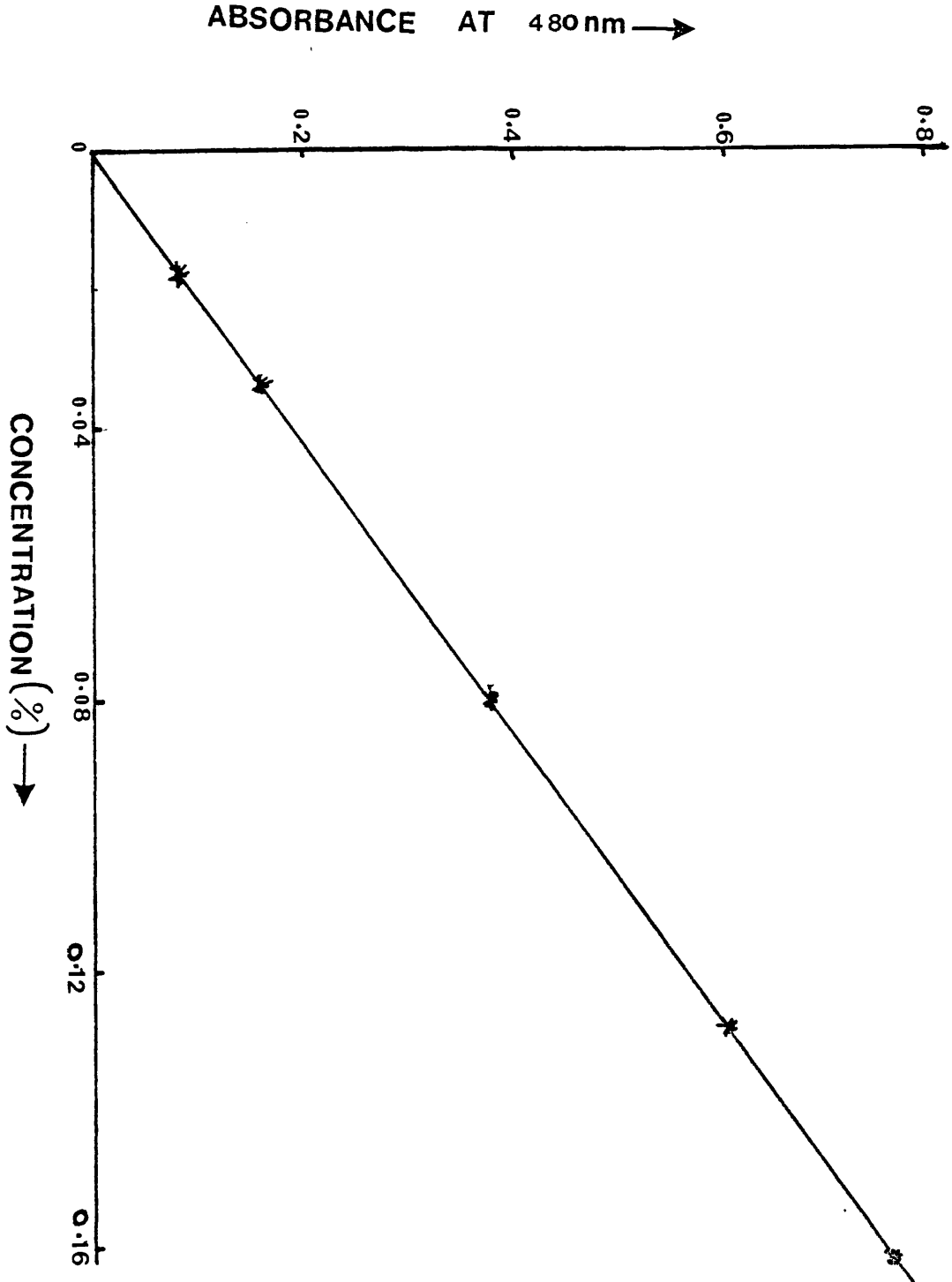


FIG. 4:7 Calibration Curve of Phenetsal / 4—Aminophenazone Colour Formation at 480 nm

Once the rate constant (k) for the benorylate degradation per hour at 25°C was known, the length of time (t_{90}) required for the 'Benoral' suspension to fall to 90% potency was obtained by application of the zero order reaction rate:

$$A_t = A_o - kt$$

where 'A_o' and 'A_t' are the initial (100%) and final (90%) potencies of the drug.

4.2.4 Prediction of the shelf life at 25°C (t_{90})

The 'Benoral' suspension gave an average shelf life at 25°C of 28 months (basing the calculation on t_{90}) Table 4:7, page 313. A more striking result obtained was the shelf life for the freshly prepared benorylate suspension in water. A shelf life of 4.9 years was obtained.

From figure 4:6, page 304, it is seen that a linear relationship between log k and 1/T was obtained for temperatures up to 60°C. There was no linear relationship at 70°C. This is in agreement with Grimshaw's report[182] on the accelerated stability testing of pharmaceutical products. In his report Grimshaw pointed out that 60°C is the highest temperature suitable for accelerated stability testing for suspensions. For coated tablets and dry tablets, the highest temperatures are 45°C and 80°C respectively.

In conclusion, it is probable that the linear relationships obtained for the absorbance *versus* time (figure 4:5, page 303) and log k *versus* 1/T (figure 4:6, page 304) graphs are an indication of the suitability of the method for the prediction of the shelf life of 'Benoral' suspension.

Table 4:1 Results for the calibration curve of phenetsal

Wt. of phenetsal taken (mg)	Concentration (% w/v)	Absorbance at 480 nm
9.2	0.0184	0.085
16.7	0.0334	0.160
40.0	0.080	0.387
65.2	0.1304	0.626
80.0	0.1600	0.769

Table 4:2 Results for the 'Benoral' suspension (Lot pH 106) - absorbances at different times for different temperatures.

30°C		40°C		50°C		60°C		70°C	
Time (hrs)	Absorbance at 480 nm	Time (hrs)	Absorbance at 480nm	Time (hrs)	Absorbance at 480nm	Time (hrs)	Absorbance at 480nm	Time (hrs)	Absorbance at 480nm
0.0	0.402	0.0	0.402	0.0	0.402	0.0	0.402	0.0	0.402
24.0	0.430	24.0	0.499	12.0	0.555	3.0	0.521	0.5	0.492
48.0	0.460	48.0	0.600	24.0	0.710	6.0	0.64	1.0	0.660
72.0	0.499	72.0	0.710	48.0	0.552	24.0	0.56	2.0	0.472
96.0	0.532	96.0	0.586	72.0	0.505	27.0	0.46	4.0	0.580
				96.0	0.550				

Table 4:3 Results for figure 4:6 (page 304)

Temp. ^{o}C	*Slope (k) (absorbance increase per hour)	log k	log k + 3	Absolute Temp. (T)	$1/T \times 10^{6}$
30	0.0014375	$\bar{3}.1574$	0.1574	303	3300.3
40	0.0043960	$\bar{3}.6431$	0.6431	313	3194.8
50	0.0129170	$\bar{2}.1113$	1.1113	323	3095.9
60	0.0396700	$\bar{2}.5985$	1.5985	333	3003.0
70	0.3360000	$\bar{1}.5263$	2.5263	343	2915.4

* Slope was obtained from figure 4:5 (page 303)

Table 4:4 Results for the freshly prepared benorylate suspension

35°C		50°C		60°C	
Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm
0.0	0.020	0.0	0.020	0.0	0.020
24.0	0.045	17.0	0.038	6.0	0.096
48.0	0.057	24.0	0.080	12.0	0.166
72.0	0.070	41.0	0.124	24.0	0.315
		48.0	0.140		

Table 4:5 Results for the stability studies of 'Benoral' suspension at different pH values at 35°C

pH 4.06		pH 5.4		pH 7.10		pH 8.0	
Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm
0.0	0.518	0.0	0.518	0.0	0.518	0.0	0.518
6.0	0.532	6.0	0.520	6.0	0.520	6.0	0.560
24.0	0.009	24.0	0.524	24.0	0.415	24.0	0.638
48.0	0.000	48.0	0.550	48.0	0.380	48.0	0.434
72.0	0.005	72.0	0.600	72.0	0.392	72.0	0.566

Table 4:6 Results for the 'Benoral' suspension buffered to pH 10.3

50°C		60°C		70°C	
Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm	Time (hr)	Absorbance at 480 nm
24.0	8.50	6.0	8.70	1.0	8.45
48.0	11.00	8.5	8.15	2.0	7.80
72.0	11.20	24.0	7.51	4.0	7.35
96.0	10.10	27.0	8.80		

Table 4:7 Results for the shelf life prediction at 25 °C

Sample	Batch No.	k at 25°C (Absorbance increase per hr)	Conc. of phenetsal produced per hr. (% w/v)	Benorylate degraded per hr. (% w/v; x 10^{-4})	Shelf life (t_{90}; months)
'Benoral' suspension	Lot pH 106	0.0009191	0.0001901	5.48	25.0
" "	Lot pH 285	0.0007482	0.0001541	4.40	31.6
Freshly prepared benorylate suspension	-	0.00009912	0.0000205	2.37	58.6

4.3 EXPERIMENTAL

Colorimetric determinations were carried out on a Sp 600 spectrophotometer series 2 and a Perkin Elmer 124 instrument.

The pH was determined using a PHM 64 Research pH meter supplied by Howe and Co.

Materials: Pure benorylate was supplied by Winthrop Laboratories, 'Benoral' suspension (3 bottles, each containing 300 ml) was purchased.

4.3.1 Synthesis of Phenetsal (*p*-Acetamidophenyl salicylate)

Salicylic acid (20 g) was treated with thionyl chloride (10.5 ml) in 1,4-dioxan (50 ml) and the mixture refluxed at 100°C for five hours maintaining an anhydrous environment with a silica gel guard tube. The excess thionyl chloride and 1,4-dioxan were removed *in vacuo*. The residue was dissolved in pyridine and added dropwise to paracetamol (21.9 g) in pyridine (20 ml) and maintained at room temperature overnight with stirring. The reaction mixture was poured into cold water (500 ml) and the precipitated solid filtered and recrystallised twice from ethanol. The phenetsal (1.5 g; 3.8%) had m.p. 186 - 188°C (lit.[193] m.p. 187°C).
δ(DMSO(d_6): 2.05 (3H, S, -COC*H*$_3$), 6.70 - 7.89 (8H, m, 8 x Ar-*H*).

4.3.2 Reagents

The 4-aminophenazone (4:4) solution: 2,3 dimethyl-4-aminophenazone (0.5 g) was dissolved in water (15 ml) and sufficient water added to produce 25 ml. The solution was protected from light.

Strong ammonia buffer: Ammonium chloride (0.675 g) was dissolved in strong ammonia (5.7 ml) and diluted with water (to 10 ml).

Dilute ammonia buffer: Strong ammonia buffer (2 ml) was diluted with water (to 1000 ml).

Potassium ferricyanide solution: Potassium ferricyanide (2 g) was dissolved in water (16 ml) and sufficient water added to produce 25 ml. The solution was freshly prepared.

4.3.3 Formulation of Benorylate suspension in water

Formula:

Benorylate	8.0 g
Methylcellulose	98
Water to	80 mls

The pH of the suspension was adjusted to 5.4 using ammonium acetate buffer[153].

4.3.4 The Kinetic studies

Aliquots (40 ml) of the 'Benoral' suspension and the benorylate suspension were maintained at different temperatures in ovens and constant temperature water baths. At various time intervals aliquots (6 ml) of the suspensions were diluted with dilute ammonia buffer (20 ml) and transferred to a separating funnel. The 4-aminophenazone solution (1 ml) and potassium ferricyanide solution (1 ml) were added to the suspension. The mixture was then shaken and the coloured derivative extracted into chloroform. The chloroform extract was passed through a cotton wool plug into a

50 ml volumetric flask and diluted to volume with chloroform. The extinction at 480 nm was measured using 1 cm cells. The concentration of phenetsal produced was obtained from a calibration curve of phenetsal at 480 nm (figure 4:7, page 305 and table 4:1, page 307). The results for the kinetic studies are given in tables 4:2 - 4:4, pages 308-310, and in figures 4:5 and 4:6, pages 303 and 304 respectively.

4.3.5 The effect of pH on 'Benoral' suspension

Aliquots (40 ml) of 'Benoral' suspension were buffered to different pH values using ammonium acetate buffer[153] and strong ammonia buffer. The buffered suspensions and an unbuffered suspension (pH 5.4) were maintained at 35°C and aliquots (6 ml) withdrawn at time intervals for analysis (section 4.3.4). The results are given in table 4:5, page 311, and in figure 4:4, page 301.

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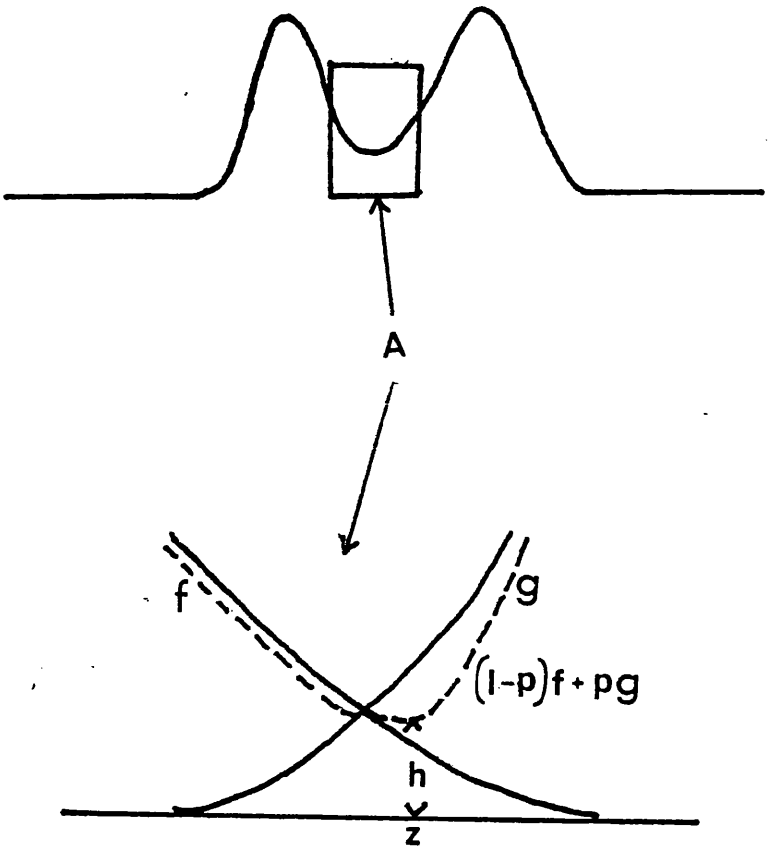
6. APPENDIX

(Mathematical Treatment for the Base Line Technique)



In the region of the minimum (A), suppose the left hand curve is $f(x)$, the right hand curve is $g(x)$; when we assume $f' < 0$, $g' > 0$, $f'' > 0$, $g'' > 0$, and f, g such that for all relevant p, $(1-p)f + pg$ has a minimum.

Suppose the minimum at Z, with minimal value h; the consequence of perturbing p to $(p+\delta p)$ is considered.

Write $\phi(x)$ for $(1-p)f(x) + pg(x)$. Thus z is governed by $\phi'(z) = 0$.

$$\text{i.e.} \quad (1-p)f'(z) + pg'(z) = 0$$

Thus to find δz, we use

$$(1-p-\delta p)f'(z+\delta z) + (p+\delta p)g'(z+\delta z) = 0$$

i.e. $$\phi'(z+\delta z) + \delta p(g'(z+\delta z)-f'(z+\delta z)) = 0$$

And expanding

$$\phi''(z)\,\delta z + O(\delta z^2) + \delta p(g'(z)-f'(z) + O(\delta z)) = 0$$

where $$\delta z = \frac{-g'(z)-f'(z)}{\phi''(z)}\,\delta p + O(\delta p^2)$$

so $$h+\delta h = (1-p-\delta p)f(z+\delta z) + (p+\delta p)g(z+\delta z)$$

$$= \phi(z+\delta z) + \delta p(g(z+\delta z)-f(z+\delta z)$$

so $$\delta h = \phi'(z)\delta z + \tfrac{1}{2}\phi''(z)\delta Z^2 + O(\delta z)^2 + \delta p(g(z)-f(z)$$

$$+ (g'(z) - f'(z))\,\delta z + O(\delta z^2))$$

$$= \{\phi'(z)\delta z + (g(z)-f(z))\,\delta p\} + \tfrac{1}{2}\phi''(z)\delta z^2$$

$$+ (g'(z)-f'(z))\delta p\delta z) + O(\,p\delta z^2, \delta z^3)$$

$$= (g(z)-f(z)\delta p + \{\tfrac{1}{2}\phi''(z)\,\frac{(g'(z)-f'(z)^2)}{\phi''(z)^2}$$

$$+ (g'(z)-f'(z)) - \frac{(g'(z)-f'(z))}{\phi''(z)}\}\,\delta p^2 + O(\delta p^3)$$

$$= (g(z)-f(z))\delta p - \tfrac{1}{2}\,\frac{(g'(z)-f(\tfrac{1}{2}))^2}{\phi''(z)}\,(\delta p)^2 + O(\delta p^3)$$

Thus δh is approximately linearly related to δp, with coefficient $g(z) - f(z)$, but the quadratic term does not vanish, and indeed the coefficient

$$-\frac{(g'(z)-f'(z))^2}{\phi''(z)}$$ need not even

be particularly small; we know $g' > 0$, $f' < 0$.